201-15015

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12/31/03 01:44 PM

To: NCIC OPPT@EPA, Rtk Chem@EPA
cc: Prakash Surana <pmsurana@celanese.com>, James Scroggins <jscroggins@celanese.com>
Subject: HPV Submission CASNO 44611-52-3

Michael O. Leavitt, Administrator

U. S. Environmental Protection Agency

P.O.Box 147

Merrifield, VA 22116

Dear Administrator Leavitt

On behalf of Celanese Limited (HPV registration number), I am submitting the attached test plan and robust summaries for "Methoxymethanol" CAS Number 4461-52-3, submitted under the United States Environmental Protection Agency's High Production Volume Chemical Challenge Program.

This document is being submitted in electronic format (Adobe Acrobat pdf file). If you require additional information or have problems with the electronic document please contact me by phone (618-539-5280) or email (erauckman@charter.net).

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Sincerely,

Elmer Rauckman PhD, DABT

Consulting Toxicologist for Celanese Limited


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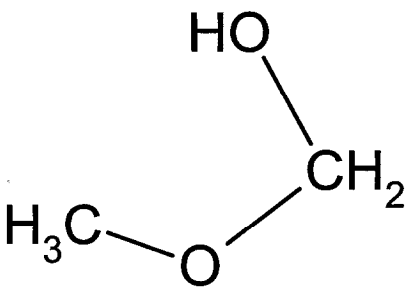
Methoxymethanol

CAS Number 4461-52-3



USEPA HPV Challenge Program Submission

Test Plan

December 28, 2003

Submitted by:

Celanese Limited

Prepared by:
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Table of Contents

Executive Overview

Methoxymethanol (CAS Number 4461-52-3) is a transient equilibrium species (or compound) found in mixtures of formaldehyde and methanol, which may also contain water. It is produced from a highly concentrated hydrated formaldehyde compound by dissolution in methanol. The primary use of these equilibrium mixtures is as a chemical intermediate in the manufacture of urea formaldehyde and melamine formaldehyde resins, which are used for coatings, adhesives, molding compounds and similar applications. These equilibrium mixtures offer advantages over using aqueous solutions of formaldehyde for many applications.

Methoxymethanol (CAS Number 4461-52-3) is not produced as a pure chemical entity as it is an unstable species that has no known application in commerce other than as an incidental species in methanolic formaldehyde. Celanese Ltd., the sponsor of this chemical in the U.S. EPA HPV Program markets a commercial product known as Methyl Formcel ® that has the nominal composition formaldehyde (55%), methanol (35%) and water (10%).

The physicochemical properties of methoxymethanol are not well established since it is not isolatable under ambient conditions. Methoxymethanol can be observed spectroscopically (e.g. nmr and ms) but is not stable enough for convenient determination of bulk physicochemical properties. The commercial product boils at between 90 and 95°C, and has a vapor pressure of 40 hPa at 40° C. Methoxymethanol, as a discrete chemical entity, is predicted to be miscible with water and have a log $K_{o/w}$ of –1.4. Physicochemical properties of formaldehyde and methanol are also described in the document as they are relevant to hazard and risk assessment of commercial product.

Methoxymethanol can be considered readily biodegradable as both methanol and formaldehyde are readily biodegradable and the half-life of the methoxymethanol molecule in water is six minutes or less. Methoxymethanol, methanol and formaldehyde undergo relatively rapid indirect photolysis in the atmosphere with half-life estimated to be less than 16 hours. EQC Level III calculations indicate that methoxymethanol, methanol and formaldehyde distribute preferentially to water and followed closely by soil.

Fish, invertebrates and algae are all predicted to be relatively insensitive to toxicity by the methoxymethanol molecule when considered as a neutral organic species; however, due to rapid hydrolysis in water, the toxicity of formaldehyde to aquatic species is considered the relevant way to evaluate the aquatic toxicity of methoxymethanol. Approximate LD_{50} and ED_{50} values have been calculated for the Celanese commercial product based on formaldehyde content to be 45, 10.5 and 11.5 mg/L for fish, invertebrates and green algae, respectively.

The acute oral toxicity in rats has been determined for a "methoxymethanol" test material that was 46.7% methoxymethanol with 44.93% methanol. The study results indicate the oral LD_{50} values for male or female rats were 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg), and 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg). These values suggest that hydrolysis to formaldehyde is the critical step in the acute toxicity of methoxymethanol. The document compares this result with the acute oral toxicity formaldehyde and methanol.

An OECD 422 guideline repeated dose with reproductive and developmental screen has been conducted on a "methoxymethanol" test material that was 46.7% methoxymethanol with 44.93% methanol. Effects appear to be primarily at the site of contact and related to the irritant properties of the test substance. The GI tract is identified as the target organ and biochemical and hematologic changes are considered secondary to gastric ulceration and subsequent loss of blood. The LOAEL was determined to be 60 mg/kg-day for males and 300 mg/kg-day for females. The NOAEL are considered to be 12 mg/kg-day for males and 60 mg/kg-day for females.

An Ames test and an in vitro chromosome aberration study have been conducted on a "methoxymethanol" test material that was 46.7% methoxymethanol with 44.93% methanol. It was found to have genotoxic activity in both assays. A discussion of the genotoxicity of methanol and formaldehyde is provided in the document.

Reproductive and developmental toxicity were assessed in the OECD-422 conducted on a "methoxymethanol" test material that was 46.7% methoxymethanol with 44.93% methanol. Although the OECD-422 results did not indicate any reproductive or developmental hazard from this mixture, developmental toxicity studies in rodents have shown specific developmental toxicity of methanol at high doses. Differences in methanol metabolism between humans and rodents indicate that these high-dose developmental effects in rodents are not relevant to man. A thorough discussion of methoxymethanol, methanol and formaldehyde is given in the document detailing the metabolic differences and evaluating the developmental hazard to man.

With regard to the parameters specified in the EPA HPV Challenge program, the available information fills all of the requirements for physicochemical parameters, environmental fate, and toxicity information. No additional testing is recommended.

Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 4461-52-3 Methoxymethanol	Information Available?	OECD Study ?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	N	N	Y	N	
Boiling Point	Y	N	N	Y	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Partition Coefficient	Y	N	N	Y	N	Y	N	
Water Solubility	Y	N	N	Y	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	Y	Y	Y	N	
Water Stability	Y	N	N	Y	Y	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	N	N	Y	N	Y	N	
Ecotoxicity								
96-Hour Fish	Y	N	N	Y	Y	Y	N	
48-Hour Invertebrate	Y	N	N	Y	Y	Y	N	
96-Hour Algae	Y	N	N	Y	Y	Y	N	
Toxicity								
Acute	Y	Y	Y	Y	N	Y	N	
Repeated Dose	Y	Y	Y	Y	N	Y	N	
Genetic Toxicology *in vitro*	Y	Y	Y	Y	N	Y	N	
Genetic Toxicology *in vivo*	Y	Y	Y	Y	N	Y	N	
Reproductive	Y	Y	Y	Y	N	Y	N	
Developmental	Y	Y	Y	Y	N	Y	N	

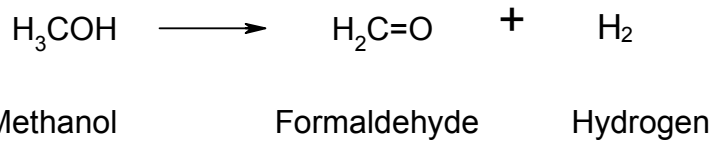
Introduction

Methoxymethanol (CAS Number 4461-52-3) is a transient equilibrium species (or compound) found in mixtures of formaldehyde and methanol, which may also contain water. Methoxymethanol (CAS Number 4461-52-3) is not produced as a pure chemical entity as it is an unstable species that has no known application in commerce other than as an incidental species in chemical mixtures that contain methanol and formaldehyde.

It is produced from a highly-concentrated hydrated formaldehyde compound by dissolution in methanol. The primary use of these equilibrium mixtures of methanol and hydrated formaldehyde is as a chemical intermediate in the manufacture of urea formaldehyde and melamine formaldehyde resins, which are used for coatings, adhesives, molding compounds and similar applications.

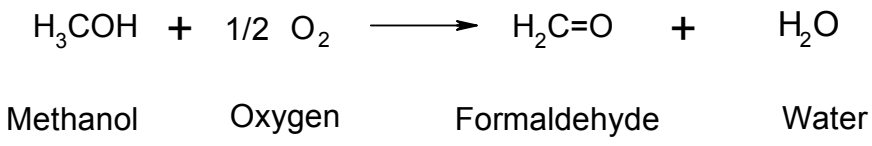
Celanese Ltd., the sponsor of this chemical in the U.S. EPA HPV Program markets a commercial product known as Methyl Formcel ® that has the nominal composition formaldehyde (55%), methanol (35%) and water (10%). The production of nominal quantities of methoxymethanol is inseparable from the commercial production of formaldehyde since methoxymethanol is produced by the reaction of formaldehyde with methanol and all commercial processes for formaldehyde production have some residual methanol. Formaldehyde is produced from methanol by two processes using either a silver catalyst or a metal oxide (iron-molybdate) catalyst. Each process is practiced in a number of variations.

The silver catalyst process is a combination oxidation-dehydrogenation of methanol and is commonly represented by the following chemical equation:



This is an exothermic reaction and the reaction is typically quenched with water and steam is recovered as a by-product. Metallic silver in the form of gauze or crystals is used as the catalyst.

The second important production method, the metal oxide process, involves the catalytic oxidation of methanol by a mixed oxide catalyst containing iron and molybdenum with other metals (e.g. chromium) often used as catalyst promoters. The reaction is typically represented as:

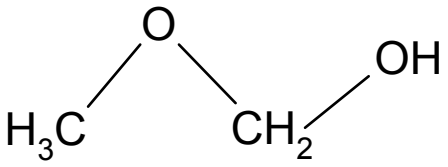


The primary use of methanolic formaldehyde (methoxymethanol) is as a chemical intermediate in the manufacture of urea formaldehyde and melamine formaldehyde resins, which are used for coatings, adhesives, molding compounds and similar applications. These equilibrium mixtures of methanol and hydrated formaldehyde offer advantages for many applications over using aqueous solutions of formaldehyde as they contain more formaldehyde per pound than aqueous preparations, they contain less water, they offer enhanced stability and they can be stored at lower temperatures than aqueous formaldehyde.

Aqueous solutions of formaldehyde require an inhibitor to prevent excessive polymerization at low storage temperatures. Methanol is most commonly used for this purpose and inhibited grades of aqueous formaldehyde usually contain 7-15% methanol. Therefore, the actual quantity of methoxymethanol in commerce is the total of the methoxymethanol contained in the methanol hydrated-formaldehyde mixtures used in industry, and the methoxymethanol contained in commercial methanol-inhibited aqueous formaldehyde.

The TSCA 1975-77 inventory reporting stated that 50,000,000 to 100,000,000 pounds of methoxymethanol were produced by one plant. Current production volumes of Methyl Formcel® are in the range of 1,000,000 to 10,000,00 pounds. Other manufactures use formaldehyde-methanol solutions as a site limited intermediate and their production level is not known. If the stabilized grades of aqueous formaldehyde are considered the annual incidental production of methoxymethanol is much greater. Although the quantity of stabilized formaldehyde produced in the US is not published it can be estimated from the annual capacity for formaldehyde production of 5,648,000 metric tons which operates at about 85% of capacity to produce about 5,000,000 metric tons of total formaldehyde. Much of this is captive and not shipped as inhibited formaldehyde. A rough estimate is that 10% of formaldehyde produced is shipped as methanol-inhibited aqueous solutions and of this, 15 mole % is in the form of methoxymethanol (*vide post*); therefore, it is possible that 150,000,000 pounds of methoxymethanol is produced, shipped and utilized in the U.S. annually as an incidental compound in formaldehyde/methanol/water ternary mixtures.

The structure of methoxymethanol is shown below:



Methoxymethanol

Methoxymethanol is also known as:

- Formaldehyde methyl hemiacetal
- Hemiformal
- Methanol, hemiformal
- Methanol, methoxy- (8CI, 9CI)
- Methyl hemiformal

The primary application of methoxymethanol is as a chemical intermediate in the manufacture of various urea formaldehyde and melamine formaldehyde resins. It is a more effective means of delivering formaldehyde to a reaction, as the quantity of formaldehyde is greater in methanol-hydrated-formaldehyde preparations than in water solutions of formaldehyde. In addition, methanol-hydrated-formaldehyde preparations have significant advantages if water is undesirable in the reaction mixture. The resins that are produced from methanol-hydrated-formaldehyde find application in a broad array of uses including coatings, adhesives, and molding compounds (1).

Celanese has a total of approximately 15–20 operators that work in areas where there could possibly be methoxymethanol exposure as a side-stream. Exposure in industrial applications is limited by process controls and protective equipment. Manufacture of this material is in a closed system and the only significant exposure is in sampling and possible rework of off-specification materials. The only other potential for exposure is in the event of a spill or upset. As the permissible exposure limit falls under the OSHA Formaldehyde Standard it is treated as a regulated chemical and when there is sampling, clean up, or rework appropriate engineering controls, work practices, and Personal Protective Equipment are specifically required for each task.

Transportation of methanol-hydrated-formaldehyde preparations is primarily in rail cars and tank trucks as the applications typically consume large quantities of this mixture and it can be conveniently piped from the transportation vehicle to storage tanks. Since the material is primarily transported by pipe in a chemical plant, and since the material falls under the OSHA Formaldehyde Standard, it is primarily handled in closed systems or with proper PPE for protection from formaldehyde when sampling, cleaning or connecting lines.

Several fate and toxicity studies have been conducted on the components of methoxymethanol (methanol and formaldehyde). These studies are briefly reviewed in this testing rationale document, which describes how they meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries or given as shorter summaries using the IUCLID format. The available data set satisfactorily fulfills the data requirements for the EPA HPV Program. Although all endpoints are not filled by direct experimental data on the methoxymethanol molecule, use of data from formaldehyde and methanol is justified as methoxymethanol is in equilibrium with these species. In addition, estimation of some endpoints from SAR relationships is necessary (due to the instability of the methoxymethanol molecule) and satisfactory to define the hazard of this mixture.

Chemistry of Methoxymethanol

It is necessary to understand some basic information about the chemistry of formaldehyde in water and alcoholic solutions to evaluate the hazards of what is known as "methoxymethanol".

Pure formaldehyde, HCHO, is a gas under normal temperatures and pressures. Commercial formaldehyde is produced as solutions in water and/or lower alcohols. Formaldehyde exists in water as an equilibrium distribution of a homologous series of hydrates such as:

Formaldehyde hydrate	HO-CH2-OH	"Methylene glycol"
Formaldehyde dimer hydrate	HO-CH2-O-CH2-OH	-
Formaldehyde trimer hydrate	HO-CH2-O-CH2-O-CH2-OH	-

etc.

In methanol, formaldehyde primarily exists as an equilibrium distribution of a homologous series of hemiacetals such as:

Formaldehyde hemiacetal	CH3-O-CH2-OH	"Methoxymethanol"
Formaldehyde dimer hemiacetal	CH3-O-CH2-O-CH2-OH	-
Formaldehyde trimer hemiacetal	CH3-O-CH2-O-CH2-O-CH2-OH	-

……..etc.

The length of these oligomers also depends on the relative proportion of formaldehyde to methanol in the mixture.

Commercially prepared aqueous formaldehyde solutions always contain a small amount of methanol (formaldehyde is produced by the partial oxidation of methanol over a metallic catalyst). In many cases, additional methanol is purposely added to the aqueous solutions as a stabilizer to allow storage at lower temperatures. Commercially prepared alcoholic solutions of formaldehyde typically contain water. For example, in Methyl Formcel®, the nominal composition is 55.0% formaldehyde, 34.5 % methanol, and 10.5% water.

Any solution containing formaldehyde, water, and methanol will be a complex equilibrium mixture of these homologous series of hydrates and hemiacetals with small amounts of "free" or uncombined formaldehyde, water, and methanol. Methoxymethanol is just one of the many equilibrium species present. Changing the temperature of the solution, the solution pH, or the concentration of any of the components will shift the equilibrium. Thus, the amount of methoxymethanol present in a formaldehyde solution is dependent not only on the nominal concentrations of formaldehyde, methanol, and water present, but the temperature and pH as well.

Maiwald, et al (2) recently studied the equilibra of these ternary mixtures of formaldehyde-water-methanol as a function of temperature between 298 and 383 K. using quantitative ^{13}C-nmr spectroscopy. They reported that in these mixtures, formaldehyde is predominantly bound in methylene glycol, poly(oxymethylene) glycols,

hemiformal, and poly(oxymethylene) hemiformals. The reader is referred to this article for an in-depth discussion of the ratios of various species as a function of temperature. Some data were extracted from the article as most useful in the current discussion about the actual nature of the methoxymethanol tested and used in industry and is presented below.

In these nmr studies (2), six ternary mixtures containing different ratios of formaldehyde, methanol and water were studied at three temperatures in two sets of independent studies in two different laboratories. As the reproducibility between laboratories was excellent and as the temperature effects were not large, the data from one laboratory at one temperature (298 °K) using two mixtures was selected for presentation. The table below gives the composition of the two mixtures studied along with the composition of the material that was tested in animals for health effects (Japanese material) and the nominal composition of the predominant commercial product (Celanese material).

Component	Materials used for nmr studies		Experimental and commercial materials	
	Mix A	Mix B	Japanese	Celanese
HCHO (mole fn)	0.2746	0.396	0.223	0.525
H_2O (mole fn)	0.6153	0.0962	0.137	0.167
MeOH (mole fn)	0.1101	0.5078	0.639	0.308

Table 1. Composition of model and commercial mixtures

From the table it can be seen that the commercial material (Celanese) is considerably higher in formaldehyde content than the other mixtures. Although the commercial material is beyond the range for interpolation of approximate quantities of the various species, the NMR data give a good indication of the actual species that exist in solution. The commercial mixture, having a higher level of formaldehyde, might be anticipated to have more F2, F3, F2Me and F4Me components and probably some F5 and F5Me and greater chain length oligomers (see table below for definition of the components). Results of the NMR determination of the relative concentrations of species are shown in Table 2.

Species		Mixture A		Mixture B	
		Peak area	Mole %	Peak area	Mole %
HO-C-OH	F1	0.1469	21.3%	0.0024	0.3%
HO-C-O-C-OH	F2	0.1699	24.7%	0.0025	0.3%
HO-C-O-C-O-C-O-H	F3	0.03	4.4%	nd	nd
HO-C-O-Me	F1Me	0.2327	33.8%	0.708	83.5%
HO-C-O-C-O-Me	F2Me	0.0805	11.7%	0.1169	13.8%
HO-C-O-C-O-C-O-Me	F3Me	0.0286	4.2%	0.0178	2.1%

Table 2. Speciation of model systems

Examination of these data suggests that methoxymethanol is a predominant component of the mixture under conditions of reduced water content and as water content increases (conditions as in the body or the environment), methoxymethanol is rapidly equilibrated into hydrated formaldehyde (F1) and hydrated formaldehyde dimer (F2). This shift in equilibrium is inferred as rapid since these experiments were conducted by mixing two binary mixtures (formaldehyde and methanol, formaldehyde and water) together and recording the spectrum soon thereafter.

Pure (100%) methoxymethanol is unstable at ambient temperatures and will readily form its own equilibrium distribution of hemiacetals, methanol, and formaldehyde. To our knowledge, pure material cannot be purchased commercially or purchased from laboratory supply houses. The limited numbers of studies that have been conducted to study the properties of pure methoxymethanol have used extraordinary conditions to isolate and stabilize the pure material for study (3, 4, 5).

Dealing with the complexity of a ternary system containing a dozen or more components may be a realistic way to examine the bulk material relative to its physical and chemical properties (e.g. vapor pressure and boiling point); however, it loses utility when considerations of systemic biological activity and environmental distributions under dilution conditions are important. Because of the dynamic equilibrium present, the mass action of biological or environmental water will effectively drive the dissociation of methoxymethanol into formaldehyde and methanol. What remains after dilution in an organism or the environment is most appropriately described as a mixture of formaldehyde and methanol; therefore, exposure of humans, animals and plants is best considered as exposure to a simple mixture of formaldehyde and methanol.

The commercial product used as a source of formaldehyde for production of resins contains as much formaldehyde as is practical, because 1.) Pure formaldehyde is difficult to transport. 2.) The maximum concentration of formaldehyde that can be prepared and stored in water at ambient temperatures is about 37% formaldehyde. 3.) Some applications for formaldehyde require removal of excess water and/or addition of methanol.

The rational for selecting the composition used in the SIDS health effects studies of methoxymethanol as conducted by the Mitsubishi Chemical Safety Institute is not available. Our understanding is that Japan had volunteered to sponsor methoxymethanol in the OECD SIDS program and had conducted the basic health effects testing before withdrawing their sponsorship of the material.

Metabolism of Methoxymethanol

Although there are no known studies of the metabolism of methoxymethanol, it can safely be assumed that the vast majority of methoxymethanol that enters the body is rapidly hydrolyzed to formaldehyde and methanol. Both of these materials are metabolized readily to formate and, if not excreted as formate, further to carbon dioxide. Some of the material also enter the C-1 metabolic pool and is incorporated physiologically into cellular macromolecules.

METABOLIC PATHWAYS

Methoxymethanol can be viewed as a mixture of two one-carbon compounds with a common ultimate metabolic product. Because of widespread use and exposure, a large body of work has been conducted on the metabolism of methanol and formaldehyde and the differences in metabolism between rodent models and humans. It is established that methanol is initially metabolized to formaldehyde in man principally by the enzyme alcohol dehydrogenase using NAD/H as a cofactor. In rodents, however, the catalase system using hydrogen peroxide as the cofactor is the primary enzyme responsible. The generated formaldehyde has a short half-life both reacting with nucleophilic molecules and being detoxified by the enzyme formaldehyde dehydrogenase to formate. The excess formate is subsequently removed from the body by urinary excretion or folate-dependent metabolism to carbon dioxide.

Although the pathway formally only requires two one-step oxidations, it is much more complicated and has both qualitative and quantitative differences between rodents and primates. The first important difference between primates (including humans) is the enzymology of methanol oxidation. In man, this oxidation is primarily completed by alcohol dehydrogenase using the plentiful NAD as the electron acceptor. In rats and mice the enzyme responsible for this oxidation is catalase, which relies on a limited flux of hydrogen peroxide as an electron acceptor. This is depicted in Figure 1.

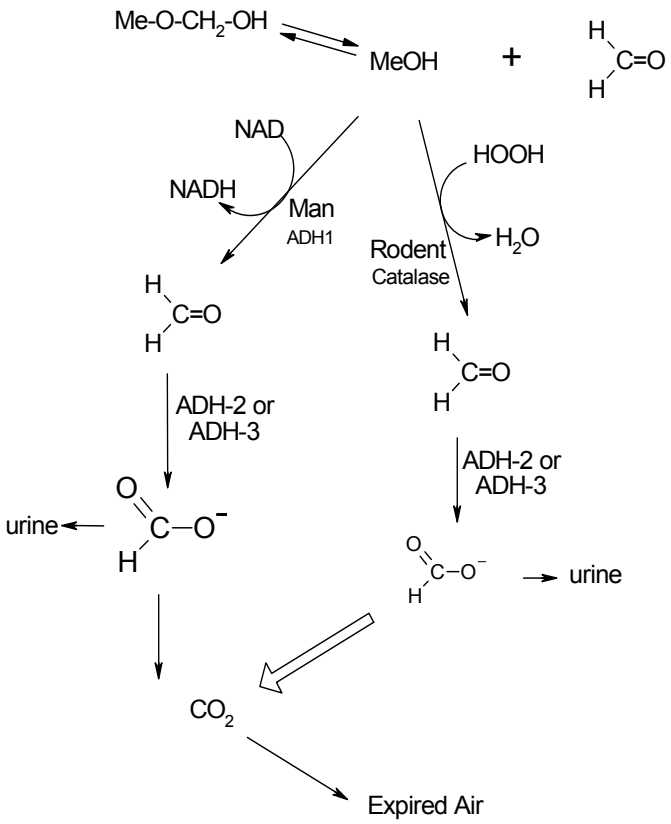


Figure 1. Metabolic Pathways for Methoxymethanol

Studies indicate the catalase pathway becomes saturated at high methanol concentrations (6) and is the rate-limiting step in rodent metabolism of methanol at high doses. In man the rate-limiting step has been shown to be oxidation of formate to carbon dioxide. Thus, at high doses of methanol rats and mice accumulate methanol while humans accumulate formate.

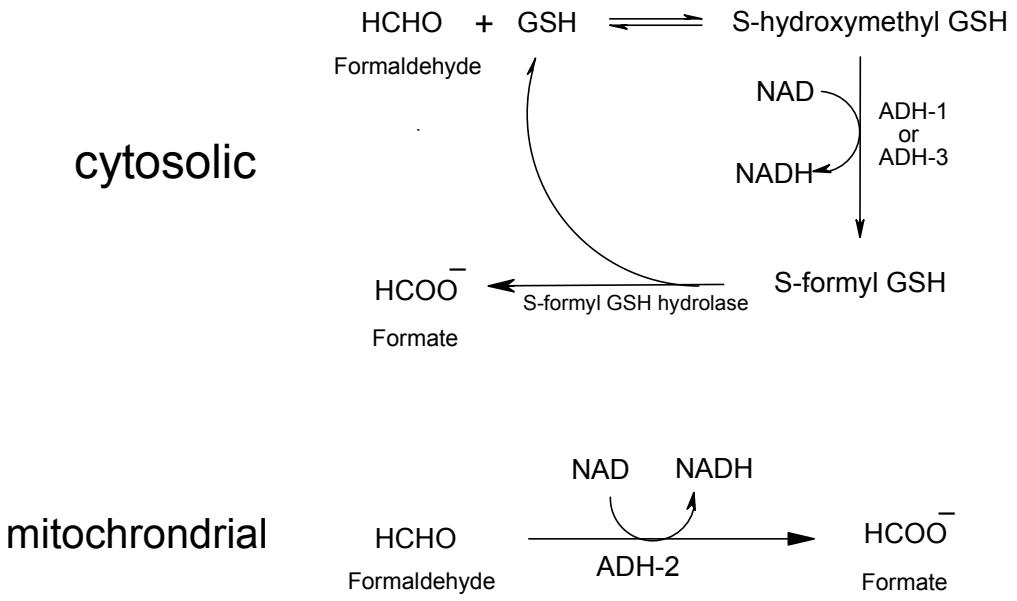


Figure 2. Metabolic Conversion of Formaldehyde to Formate

Conversion of formaldehyde to formate competes with reaction of this substance with nucleophilic centers of cells and tissues. Apparently the formaldehyde dehydrogenase system is very efficient at controlling the level of formaldehyde in the body as the half-life of formaldehyde is given as approximately one minute and the generalization can be made that formaldehyde does not accumulate in humans or experimental animals exposed to methanol (NTP CERHR). The enzyme system known as formaldehyde dehydrogenase is active in both the mitochondria and the cytosol. The cytosolic form is dependent on reduced glutathione to chemically react with cellular formaldehyde and the intermediate product, the S-hydroxymethyl GSH, is oxidized to S-formyl GSH. This S-formyl GSH is broken down into formate and reduced glutathione by S-formyl glutathione hydrolase.

Formate is a relatively unreactive product that is eliminated partly by urinary excretion but, more importantly, can be oxidized to carbon dioxide and eliminated from the lungs with expired air. This oxidation is dependent of tetrahydrofolate and in mediated by two enzymes. The first is formyl THF synthetase that catalyzes the formation of 10-formyl-tetrahydrofolate from formate and tetrahydrofolate and is dependent on a sufficient supply of tetrahydrofolate. The conversion of 10-formyl-tetrahydrofolate to carbon dioxide and tetrahydrofolate is catalyzed by formyl-THF-dehydrogenase, which also recycles the tetrahydrofolate. Tetrahydrofolate level has been established as the rate limiting cofactor in this oxidation for primates, and as rodents have higher levels of tetrahydrofolate than primates, there is a build up of format in primates that is not typically observed in rats and mice.

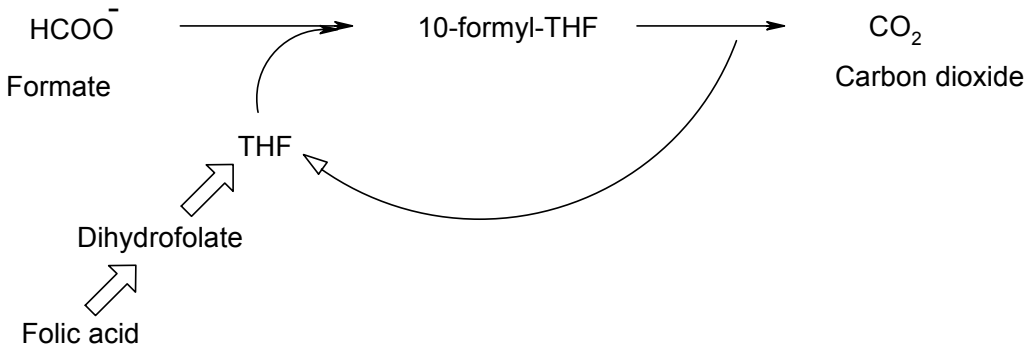


Figure 3. Metabolism of Formate to Carbon Dioxide

It is generally accepted that the known typical human ocular effects (blindness) that result from overexposure to methanol is produced by formic acid (acidosis) and formate toxicity. This effect is not seen in rodents unless the metabolic conversion of formate to carbon dioxide is reduced (e.g. folate deficiency).

These marked metabolic differences between rodents and primates suggest that the rodent is not a satisfactory effects model for methoxymethanol. These differences have also been advanced as reasons to be cautious in hazard assessment of methanol regarding developmental toxicity (6, 7).

Physico-chemical Data

Physical-chemical data for commercial product containing methoxymethanol are available from the manufacturer's information but it must be emphasized that this is a mixture that can vary in composition and definitive physical-chemical data are not appropriate.

	Formcel® Commercial Product ("methoxymethanol")	Formaldehyde	Methanol
Melting Point	NA	-92˚ C (8)	-97.8° C (8)
Boiling Point	90-95°C@1013 hPa (9)	-19.5°C @1013 hPa (8)	64.7°C @1013 hPa (8)
Vapor Pressure	90-95 hPa @ 40˚C (9)	5174 hPa @ 25 (10)	169 hPa @ 25 (10)
Partition Coefficient	Log $K_{o/w}$ = -1.4 (11)*	Log $K_{o/w}$ = 0.35 (12)	Log $K_{o/w}$ = -0.77 (12)
Water Solubility	Soluble in all proportions (9)	~ 55 weight %	Soluble in all proportions (8)
* Calculated value for pure methoxymethanol			

Table 3. Physicochemical Data for Methoxymethanol, Formaldehyde and Methanol

The physical properties of the molecular species methoxymethanol can be estimated using EPIWIN. The estimated boiling point is 91°C and the estimated vapor pressure is 43 hPa at 25°C. These measured and calculated properties indicate that methoxymethanol and commercial mixtures of formaldehyde and methanol are volatile liquids with high water solubility. The value of the partition coefficients suggests that all of the major components will partition preferentially into water and have little potential for bioaccumulation.

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints.

Environmental Fate and Pathways

Biodegradation potential was determined using various methods for both formaldehyde and methanol. There is a solid body of experimental evidence indicating that both can be considered readily biodegradable according to the OECD criteria (13). Considering that methoxymethanol is in equilibrium with formaldehyde and methanol, as methanol and formaldehyde are removed from solution by biodegradation the equilibrium drives hydrolysis of more methoxymethanol to formaldehyde and methanol, especially in dilute aqueous solutions. Thus, even if bacteria do not effectively attack the methoxymethanol molecule, its biodegradation will be facile due to its equilibrium with readily biodegradable formaldehyde and methanol. The existence of a variable quantity of paraformaldehyde in the mixture is another consideration. As paraformaldehyde breaks down in water solution to formaldehyde (14), it will be biodegraded readily after hydrolysis. The breakdown of paraformaldehyde will in turn be dependent on its initial concentration, dissolution rate, temperature and pH. For the purpose of the HPV program, its contribution to the fate of methoxymethanol preparations is suffiently understood.

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals and was used to estimate the indirect photodegradation rates of the mixture. Direct photolysis, however, was considered first as it is important when it can occur. Before conducting the calculations, consideration has to be given to the nature of the material in the atmosphere.

As we are dealing with variable composition commercial mixtures and assuming the vapor phase chemistry of the various components is similar to the liquid phase chemistry, it can be surmised that there will be four primary components introduced into the atmosphere resulting from the use of commercial methoxymethanol. These are methoxymethanol, formaldehyde, methanol and water. An important secondary component would be the hydrated form of formaldehyde (dihydroxymethane) resulting from the reaction of formaldehyde with atmospheric water vapor. In liquid phase this hydration is a very facile reaction with an equilibrium constant greater than 1000 favoring the hydrated form (15). Formation of polymeric forms of formaldehyde in the atmosphere is not considered important due to dilution effects in the vapor phase; however, sublimation of oligomeric formaldehyde from spills of commercial methanol-hydrated-formaldehyde is possible. The APOWIN calculation (shown in the robust summaries) indicates that hydrogen abstraction is very a favorable process for

reaction of oligomeric formaldehyde with hydroxyl radical and oligomers will only have an atmospheric half-life of approximately two hours. Thus, as oligomers are expected to contribute little to the quantity of material in the air and will not contribute to an extended half-life, they can be ignored relative to atmospheric photodegradation.

Regarding direct photolysis, it can be surmised by inspection of the possible species formed in the atmosphere that none of these has a chromophore that absorbs light above 295 nm (the approximate cut off for light energy transmitted to the troposphere). Direct photolysis, therefore, is considered unimportant.

In figure 4 the chemistry of methoxymethanol in the atmosphere is shown with half-lives for photodegradation that were calculated by APOWIN.

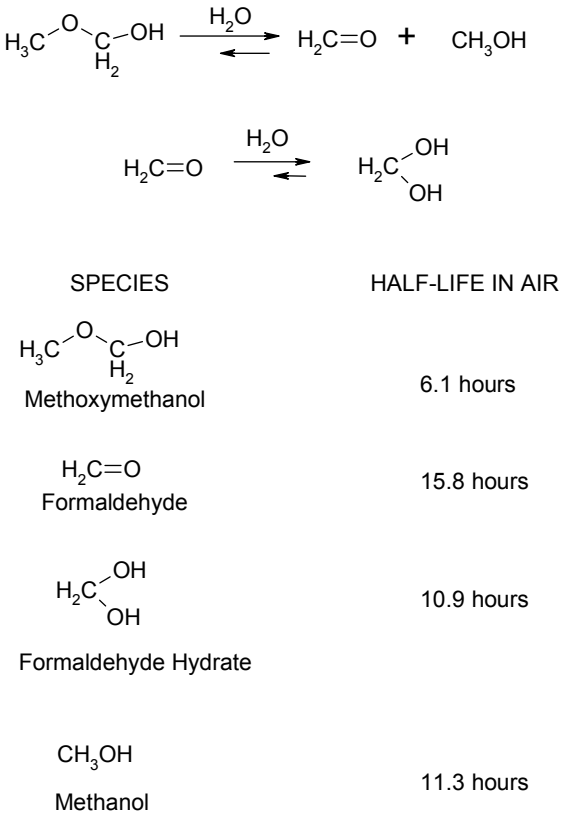


Figure 4. Atmospheric Chemistry and Photodegradation

APOWIN estimates the rate constant for reaction of a substance with hydroxyl radicals that formed in the troposphere. The program uses the estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced an estimated rate constant for each atmospheric component. Using the default atmospheric hydroxyl radical concentration in APOWIN (1,500,000 molecules/cc) and the estimated rate constant for reaction of each material

with hydroxyl radical, the estimated half-life of each component was calculated. These half-life values are shown in Figure 4. Because methoxymethanol as produced and used (methanol-hydrated-formaldehyde) has a variable composition, and as the amount of atmospheric water vapor affects the chemistry, only a range of half-live values can be given. Fortunately, all components have similar hydroxyl radical reaction rate constants and the range is reasonably narrow. The half-life range is estimated to be 6.1 to 15.8 hours (see accompanying robust summary).

Water stability has been quantitatively determined for methoxymethanol in a series of kinetic studies. The second order rate constants for reaction of methoxymethanol with water, hydrogen ion and hydroxide ion have been determined at 25°C (16). To estimate the half-life in water at various pH levels, these second-order rate constants were converted to pH specific pseudo first order rate constants and the half-lifes calculated for the water, the hydrogen ion and the hydroxyl ion reaction at several pH levels. From these calculations, the half-life of methoxymethanol at various pH levels can be determined. As there is also a dependency of the hydrolysis rate on ionic strength and the nature of the solutes in natural waters, these half-lives must be viewed as approximations of the actual hydrolysis rate under environmental conditions. The maximum half-life is about six minutes and is shortened by acidic or basic conditions but displays a broad peak (see robust summary for actual rate constants).

pH	$T_{1/2}$ Water	$T_{1/2}$ H+	$T_{1/2}$ OH^-	Approximate overall half-life
2	6 min	2 min	810 hours	2 min
4	6 min	3.3 hours	81 hours	6 min
6	6 min	333 hours	490 min	6 min
7	6 min	>1000 hours	49 min	6 min
8	6 min	>1000 hours	4.9 min	5 min
9	6 min	>1000 hours	30 sec	30 sec

Table 4. The Half-life of Methoxymethanol in Water at Several pH Values

Methanol is a simple alcohol and alcohols are one of the chemical groups considered stable to hydrolysis (17). Methanol, therefore, is considered to be a water stable component of this mixture.

Formaldehyde is known to be water reactive reversibly forming a hydrate (HO-CH2-OH) the equilibrium constant for formaldehyde hydrate formation is > 1000 (18). Thus, formaldehyde is known to be stable indefinitely in water existing 99.9% as a hydrated species.

Theoretical Distribution (Fugacity) of methoxymethanol in the environment is an extremely complicated matter due to the atmospheric chemical reactions and equilibrium of methoxymethanol with formaldehyde and methanol that the equilibrium between formaldehyde and its hydrate. These equlibria are dependent on atmospheric water vapor concentration and temperature.

To simplify matters a Level 3 fugacity model was run on each major component independently using the MacKay model with standard defaults in EPIWIN v 3.05. Actual physical properties were used for methanol and formaldehyde. Because methoxymethanol, to our knowledge, has never been studies in the pure bulk liquid form, the EPIWIN calculated values were accepted for the fugacity calculation.

Media	Methoxymethanol	Methanol	Formaldehyde
o Air	1.92%	13%	2.7%
o Water	54.8%	47.2%	51.3%
o Soil	43.2%	39.7%	45.9%
o Sediment	0.0913%	0.0705%	0.0871%

Table 5. Level 3 Fugacity Calculations

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints.

Ecotoxicity

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to aquatic species (34), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to aquatic species, formaldehyde is the species that will determine the acute ecotoxicity of this mixture. In recognition of this, the robust summaries flagged as "critical for SIDS endpoint" have been adopted from the formaldehyde SIDS document. The values for formaldehyde were adjusted to account for the 55% concentration of formaldehyde in commercial product and the adjusted vales are presented below. Full details and robust summaries for the critical studies are presented in the attachment (Robust Summary Document). The reader is also referred to the formaldehyde SIDS document (19) for more supporting studies.

Acute Aquatic Toxicity of Methoxymethanol	
Fish, 96 hour LC_{50}	ca. 45 mg/L
Daphnia, 48 hour EC_{50}	ca. 10.5 mg/L
Algae, 72 hour EC_{50}	ca. 11.5mg/L

Table 6. Acute Aquatic Toxicity of Methoxymethanol

As part of the estimation procedure, to gain some assurance that the methoxymethanol molecule did not have higher toxicity potential that formaldehyde, ESOSAR estimates for the toxicity of methoxymethanol were made using the neutral organics model. These are presented below:

Species	Duration	Endpoint	Prediction mg/L
Fish	96-hr	LC_{50}	72256.13
Fish	14-day	LC_{50}	76256.13
Daphnid	48-hr	LC_{50}	61217.39
Green Algae	96-hr	EC_{50}	31468.4
Fish	30-day	ChV	5381.146
Daphnid	16-day	EC_{50}	709.373
Green Algae	96-hr	ChV	441.037
Fish (SW)	96-hr	LC_{50}	3197.973
Mysid Shrimp	96-hr	LC_{50}	2.36E+05
Earthworm	14-day	LC_{50}	4256.716

Table 7. ECOSAR Predictions for Methoxymethanol

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints without unnecessary aquatic animal usage.

Health Effects

Limited health effects studies that were designed specifically to fill the SIDS HPV endpoints have been conducted using methoxymethanol. These studies were recently conducted at the Kashima Laboratory of the Mitsubishi Chemical Safety Institute Ltd. (20). These studies used a material that was 46.7% HCHO with 44.93% Methanol (essentially a two to one molar ratio of methanol to formaldehyde).

In addition, a very comprehensive set of studies has been conducted to determine the potential health effects of both formaldehyde and methanol. From a health effects data view, these are two of the best-studied materials in commerce. As methoxymethanol will readily breakdown in the environment and the body, formaldehyde and methanol data are also relevant to the toxicity of this commercial material.

NOTE: In the following discussion the term "methoxymethanol" will generally be used to refer to the materials actually being tested but should be kept in mind that the methoxymethanol molecule is in dynamic equilibrium with formaldehyde, methanol and water.

Acute Toxicity

Oral Exposure

A modern guideline or guideline-like oral-gavage study has been completed by the Kashima Laboratory of the Mitsubishi Chemical Safety Institute Ltd. (21). This study used a test material that was 46.7% methoxymethanol with 44.93% Methanol and the remainder presumed to be water. The study found the oral LD_{50} values for male or female rats were 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg), and 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg). Full details are given in the accompanying robust summary.

The acute oral LD_{50} of formaldehyde has been reported to be 600-700 mg/kg by Tsuchiya K. *et al.* (22) and 800 mg/kg by Smyth *et al*. (23). In the studies, Wistar rats were treated by gavage with 2 or 4 % formaldehyde solutions (formaldehyde with or without methanol stabilization). No relevant differences in toxicity were observed with regard to the additional methanol. Lethality occurred mainly during the first day after administration. Signs of toxicity were not reported.

The acute oral toxicity of methanol to rats has been reported several times in the literature. LD_{50} values of 5628 mg/kg (24), 9100 mg/kg (25), 9470 mg/kg (26), 11520 mg/kg (27), and 12750 mg/kg (28) have been reported. Although many of these reports lack details, the approximate range is consistent.

The oral LD_{50} of methoxymethanol as prepared in a methanolic solution was found to be about 1350 mg/kg. As discussed in the chemistry section, this mixture is about 25% formaldehyde. If the LD_{50} is corrected to a

formaldehyde basis, the corresponding LD_{50} would be 350 mg/kg. As the reported oral LD_{50} for formaldehyde is 600-800 mg/kg, this implies that there is some additional acute toxicity due either to the methanol or the existence of the formaldehyde as methoxymethanol. As the acute toxicity of methanol is an order of magnitude less than that of methoxymethanol mixture (as tested) or formaldehyde in aqueous solution and as methanol is metabolically converted to formaldehyde and then formate, it seems unlikely that there is a joint systemic toxic action of formaldehyde and methanol. It is speculated that the presence of excess methanol in the gavage solution has a stabilizing effect on the formaldehyde, which reduces its covalent bonding to the gastric mucosa and facilitates systemic absorption of formaldehyde. Although this is purely speculation, it is consistent with the known acute oral toxicities of formaldehyde and methanol (including the metabolism of methanol to formaldehyde), the known high reactivity of formaldehyde, the known stabilizing effect of methanol on formaldehyde solutions and the known chemical equilibrium state of ternary mixtures of formaldehyde, methanol and water (*vide ante*).

What is clear is that the rat oral LD_{50} of methoxymethanol as prepared is not greatly different from what would be predicted by considering methoxymethanol to be a simple mixture of formaldehyde, methanol and water. Based on this knowledge, the approximate LD_{50} of any commercial ternary mixture of formaldehyde, methanol and water (the commercial product know as methoxymethanol) can be predicted based on the formaldehyde content and adding an uncertainty factor in the range of two to account for either joint toxic action or facilitated absorption.

Rat Acute Oral Toxicity Data			
Material	**LD_{50} (mg/kg)**		**Reference**
	Male	Female	
Methoxymethanol with Methanol	1269	1451	21
Formaldehyde	600-800 mg/kg		29
Methanol	5,600-12,000 mg/kg		24, 25, 26, 27, 28

Table 8. Acute Oral Toxicity Data

Inhalation Exposure

No data were found for the inhalation toxicity of methoxymethanol. From a practical viewpoint, this material will dissociate into formaldehyde and methanol in moist air. As it is unstable, formaldehyde acute inhalation toxicity would appear to provide a reasonable surrogate.

The 4-hour acute inhalation LC_{50} of formaldehyde has been reported to be 480 ppm by Nagorny *et al.*(30) and the 4-hour LC_{50} for methanol has been reported as 64,000 ppm (31) and 73,000 ppm (32).

Dermal Exposure

No studies of the acute dermal toxicity of methoxymethanol were found.

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although this material is variable in composition the high level of acute toxicity for formaldehyde provides a reasonable estimate for oral and inhalation toxicity.

Repeat Dose Toxicity

Oral Exposure

A modern guideline OECD-422 repeated-dose study with a reproductive and developmental screen has been conducted on methoxymethanol by the Kashima Laboratory of the Mitsubishi Chemical Safety Institute Ltd.

This OECD-422 study was conducted using Sprague-Dawley rats the same test material that was employed in the acute oral study and the two genotoxicity studies. The test material was 46.7% methoxymethanol with 44.93% methanol and the remainder presumed to be water. Doses were selected as 12, 60 or 300 mg/kg-day based on a preliminary study. Dosing was started 14 day prior to mating and continued until day four of lactation for the dams (4 to 47 days) and for a total of 44 days for the males. Pregnant females were allowed to litter and the pups were thoroughly examined at littering and on lactation day four, when they were sacrificed. Complete necropsies were performed on parental animals followed by microscopic examination of tissues. Hematology and clinical chemistry studies were also conducted.

The major effect of methoxymethanol on parental animals was severe irritation of the gastric musosa. Which was revealed most clearly by the microscopic examination Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300 mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of

basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border. Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls. Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

Hematology revealed changes in RBC's (reduced number), reticulocytes and platelets (increased) that were only seen in the high-dose males. These effects may have been related to gastric ulceration and subsequent loss of blood. Clinical chemistry revealed effects only for the high-dose males. Effects were confined to reduction in total protein and albumin and the albumin/globulin ratio. These effects are consistent with gastric ulceration and subsequent loss of blood and are considered secondary to the gastric lesions.

Effects appear to be primarily at the site of contact and related to the irritant properties of the test substance. The GI tract is identified as the target organ and biochemical and hematologic changes are considered secondary to gastric ulceration and subsequent loss of blood. The LOAEL was determined to be 60 mg/kg-day for males and 300 mg/kg-day for females. The NOAELs are considered to be 12 mg/kg-day for males and 60 mg/kg-day for females.

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points: generally one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of this material, adequate "*in vitro"* and *"in vivo"* tests have been conducted to cover both of these two endpoints and, in addition, exhaustive genotoxicity studies have been conducted for both Formaldehyde and Methanol.

Genetic Toxicology *in vitro*

A Salmonella reverse mutation assay is available on methoxymethanol and used a material that was 46.7% methoxymethanol with 44.93% Methanol and the remainder presumed to be water. In this study methoxymethanol was found to have activity toward strains TA98 and TA100. The activity of methoxymethanol was marginal but it was strong enough to meet the criteria of a doubling of the number of revertant colonies seen in the controls and a dose-response relationship. As expected for this formaldehyde releasing material, methoxymethanol was toxic to Salmonella at 500 μg/plate and above.

These revertant results are both qualitatively and quantitatively similar to the results obtained by the NTP (33) using formaldehyde. In the NTP study results, toxicity was observed in the range of 166 to 333 μg/plate and the

maximum genotoxic activity was observed at correspondingly lower concentrations than methoxymethanol. This is consistent with the hypothesis that in water solutions the methoxymethanol equilibrium with formaldehyde and methanol strongly favors the hydrolyzed forms of free formaldehyde and methanol.

Studies have shown that methanol is negative in bacterial reverse mutation assays and is essentially non-inhibitory to test Salmonella and *E. coli* (34). These data on methoxymethanol suggest that in aqueous solutions, methoxymethanol acts like a simple Formaldehyde solution and that the methanol (both the stoichiometric excess and that produced by hydrolysis of methoxymethanol) does not influence either the mutagenic properties of formaldehyde toward sensitive bacterial strains nor the cytotoxicity of formaldehyde toward these bacteria.

Genetic Toxicology *in vivo*

Mammalian genotoxicity was assessed "*in vivo*" using Chinese hamster lung (CHL) cells in an *in vitro* chromosomal aberration test with methoxymethanol. The test material was the same that was used for the bacterial reverse mutation assay and was 46.7% methoxymethanol with 44.93% methanol and the remainder presumed to be water.

After exposing CHL cells for 24 hours to methoxymethanol, the proportion of cells with chromosomal structural changes and polyploid cells increased significantly in a concentration-related fashion. In the high concentration group (0.020 mg/ml), methoxymethanol was determined to be positive for structural aberrations and equivocal for polyploid cells.

After exposing CHL cells for 48 hours to methoxymethanol the proportion of cells with chromosomal structural changes and polyploid cells were significantly increased and indicated a equivocal result high concentration group (0.020 mg/ml). On the other hand, the high concentration group with metabolic activation with 6-hour exposure showed chromosomal structural aberrations in more than 16% of the cells examined indicating a positive result. The frequency of polyploid cells increased significantly in the medium and high concentration groups indicating equivocal results.

It is concluded that methoxymethanol is positive for producing chromosomal structural aberrations in CHL cells in vitro. This result is similar to that obtained for formaldehyde using CHO cells where it was found to be positive for producing chromosome aberrations in the presence or absence of metabolic activation at concentrations levels similar to those producing positive results for methoxymethanol (35).

Methanol, on the other hand, is considered non-genotoxic and of low cytotoxicity (36). As was the case for the bacterial reverse mutation assays, these data on methoxymethanol suggest that in aqueous solutions, methoxymethanol acts like a simple Formaldehyde solution and that the Methanol (both the stoichiometric excess

and that produced by hydrolysis of methoxymethanol) does not influence either the genotoxic properties of Formaldehyde toward sensitive mammalian cells nor the cytotoxicity of Formaldehyde toward these cells.

Recommendation: The HPV requirement for genetic testing has been met as assays sensitive to both point mutation and to clastogenic effects have been conducted using an acceptable protocol. No additional testing is recommended.

Reproductive Toxicity

Methoxymethanol was tested for reproductive toxicity using a combined repeat-dose, reproductive and developmental toxicity screening study (OECD 422). Please refer to the "repeated dose" section of this document or the accompanying "robust summary" for more information about the study design. All females, where mating was confirmed, became gravid. There was no effect of the test substance on either the mating or fertility indices. Most pairs successfully mated during the first estrous cycle and there were no significant differences among groups on the day of mating. There was no difference between control and dosed groups on any parameter associated with successful mating, gestation or delivery. Histopathological examination of the parental generation revealed no adverse effects on the reproductive organs.

Although this is only considered a screening study, no hints of adverse reproductive effects were reported. In addition, both Formaldehyde and Methanol have been investigated regarding reproductive toxicity. No adverse effects of chronic formaldehyde administration by drinking water on reproductive organs were reported in an 1989 in a chronic study with rats by Til et al. at doses that induced stomach lesions (approx. 82 and 109 mg/kg-day for male and female rats, respectively). Ovaries and testes of a subset of animals (at least 10 animals per dose and gender) were weighed at weeks 53, 79 and 105. Histological examinations of ovaries, mammary glands, uteri and testes, prostate glands, epididymides were performed on all animals of control and high-dose groups. Mammary glands, ovaries and testes of three low- and mid-dose group animals were also examined in week 105 (37).

Potential adverse effects of methanol on reproductive organs were studied by Lee et al. (38), who exposed 8-week-old Sprague-Dawley rats at 200 ppm for 8 hours/day (ca. 37 mg/kg) for 1– 6 weeks and observed no effect on testosterone levels, weights of androgen sensitive organs, capability of in vivo-exposed testes to produce testosterone in vitro; he also reported lack of gross morphological effect on reproductive organs. In addition, normal and folate-deficient, Long-Evans rats exposed to 800-ppm methanol for 20 hours/day (ca. 378 mg/kg-day), 7 days per week for 13 weeks had no adverse findings in the testicular histology at 10 months of age (ibid.). A study reported by Poon et al. (39) reported no lesions in the reproductive organs of 4–5 week-old male and female Sprague-Dawley rats that inhaled 2,500-ppm methanol vapors for 6 hours/day (ca 370 mg/kg-day) for 4 weeks. Overall, the weight of evidence indicates little potential for methanol-induced adverse reproductive effects.

In summary, a combination of studies on methoxymethanol, formaldehyde and methanol indicate no adverse effects on reproduction or reproductive organs.

Recommendation: No additional testing is required as the available data are sufficient to assess the reproductive toxicity of this material.

Developmental Toxicity

Methoxymethanol was tested for reproductive toxicity using a combined repeat-dose, reproductive and developmental toxicity screening study (OECD 422). Please refer to the "repeated dose" section of this document or the accompanying "robust summary" for more information about the study design.

In brief, results of the developmental toxicity aspects of this OECD-422 study showed that no malformations were observed that were attributable to administration of the test substance. High-dose pups were not different from controls in body weight, sex ratio, mean pup weights, number of pups born, or other similar parameters. Skeletal examination of the high-dose and control groups showed no compound-related effects. Visceral examination revealed a significant increase in the occurrence of patent foramen ovale in the 300 mg/kg-day group. This is interpreted as a fetotoxic effect at the high dose associated with a developmental delay. The developmental and maternal NOAEL is considered to be 60 mg/kg-day.

The developmental toxicity of formaldehyde has been studied in an inhalation prenatal toxicity study reported by Martin (40,41). This study indicated no teratogenic effects after inhalation of 2, 5, or 10 ppm (2.4, 6, 12 mg/m3; ca 0.23, 0.65 or 1.3 mg/kg-day) formaldehyde during gestation days 6 - 15 in the rat. There were two control groups in the study, one was sham-treated (air only), and the other was maintained without any treatment in the animal room. At 10-ppm formaldehyde, a significant decrease in maternal food consumption and body weight gain was reported but pregnancy parameters were unaffected. No evidence of maternal toxicity or developmental that was considered related to exposure was found at the lower concentration levels. The maternal NOAEL is 5 ppm and the fetal NOAEL is 10 ppm. These results are supported by a teratogenicity study by Saillenfait et al. reported in 1989 (42), in which higher formaldehyde concentrations (up to 40 ppm, 50 mg/m^3) were used for exposure. At 20 ppm (25 mg/m^3) and above a slight decrease of the fetal weights was observed. These concentrations, however, cause severe irritations of the upper respiratory tract of dams.

Several developmental toxicity studies of methanol have been conducted and it has been reported that methanol is a developmental toxin to rodents at very high doses. In a study reported by Nelson et al. (43) inhalation exposure of Crl:Sprague-Dawley rats to 20,000 ppm methanol vapor for 7 hours/day (ca. 3,300 mg/kg-day) on gestation day 7–15 was associated with prenatal developmental toxicity evidenced by reduced fetal weight, increased litter incidence of exencephaly and encephalocele, and skeletal malformations. This inhalation concentration also caused clinical signs of maternal intoxication in the early days of exposure but no other maternal effects were reported. Developmental toxicity without malformations was also observed following exposure to 10,000 ppm for

7 hours/day (ca. 1650 mg/kg-day) on gestation day 1–19 as evidenced by statistically significant reductions in fetal body weight. The National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (CERHR) panel's review of this study designated 10,000 ppm inhaled methanol as a maternal NOAEL and 5,000 ppm as a fetal NOAEL (44)

In mice, positive studies were reported by Rogers et al. (45). In these studies, exposure of Crl:CD-1 mice to methanol vapor at concentrations of 2,000 ppm or greater for 7 hours/day on gestation day 6 –15 was reported to be associated with developmental toxicity as evidenced by cleft palate, exencephaly and skeletal malformations. The initial appearance of malformations was reported to be cervical ribs seen at 2,000 ppm and cleft palate and exencephaly at 5,000 ppm, and adverse effects on the number of live pups per litter and fetal weight were seen at 7,500 and 10,000 ppm, respectively. No maternal toxicity was apparent at any dose and the developmental NOAEL was considered to be 1000 ppm. Additional studies were conducted by gavage to link the inhalation produces blood methanol levels with blood methanol levels produced by gavage. They established that twice-daily gavage dosing with 2000 mg/kg (4,000 mg/kg-day) produced blood levels similar to those produced by inhalation exposure at 10,000 for 7 hours/day. In addition, this twice daily gavage dosing produced similar a similar pattern of developmental as the 10,000 ppm 7 hour/day inhalation exposure.

It should be kept in mind when evaluating the developmental data relative to human risk that there are multiple differences in the way methanol is detoxified in a rodent verses a human. These differences, discussed in detail in an earlier section of this document, indicate that additional caution should be used when extrapolating rodent data from methanol to humans as findings in rodents may not be directly applicable to man.

Recommendation: No additional testing is required as the available data are sufficient to assess the developmental toxicity of this material.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, the available information fills all of the requirements for physicochemical parameters, environmental fate, and toxicity information. No additional testing is recommended.

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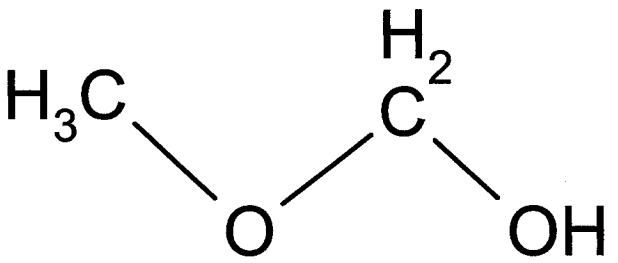
201-15015B

Methoxymethanol

CAS Number 4461-52-3



HPV Data Set

Existing Chemical	:	ID: 4461-52-3
CAS No.	:	4461-52-3
EINECS Name	:	methoxymethanol
EC No.	:	224-722-2
Molecular Formula	:	C2H6O2

Producer related part

Company	:	Celanese Ltd
Technical Contact		Prakash Surana Celanese Ltd. P.O. Box 819063 Dallas, TX 75381 pmsurana@celanese.com (972) 443-4836

Prepared by: Toxicology and Regulatory Affairs, Freeburg IL
CONTACT INFO: Elmer Rauckman (618-539-5280)
rauckman@toxicxolutions.com

Substance related part

Company	:	Celanese Ltd
Creation date	:	20.08.2003
Printing date	:	31.12.2003
Revision date	:	
Date of last update	:	23.12.2003
Number of pages	:	46

1.0.1 APPLICANT AND COMPANY INFORMATION

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23.12.2003

1.2 SYNONYMS AND TRADENAMES

Formaldehyde methyl hemiacetal

20.08.2003

Hemiformal

20.08.2003

Methanol, hemiformal

20.08.2003

Methanol, methoxy- (8CI9CI)

20.08.2003

Methyl hemiformal

20.08.2003

2.1 MELTING POINT

Remark :
There is no defined melting/freezing point for this mixture.
At temperatures below 65 deg. C, solid polymeric formaldehyde gradually forms.
At temperatures below 0 deg. C, ice crystals can form

In the environment the material will readily dissociate to:
Formaldehyde with a melting point of -92 deg. C (Merck Index, 13th Edition)
Methanol with a melting point of -97.8 deg C (Merck Index, 13th Edition)

Test substance :
Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

Reliability : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a variable mixture

Flag : Critical study for SIDS endpoint
22.11.2003 (8)

2.2 BOILING POINT

Value : ca. 90 - 95 °C at 1013 hPa

Remark :
The boiling point will vary depending on the exact composition of the mixture. The range given is for the specified mixture. As other compositions of this mixture may be sold, this range may not be universally valid

In the environment the material will readily dissociate to:
Formaldehyde with a boiling point of -19.5 deg. C @1013 hPa (Merck Index, 13th Edition)
Methanol with a boiling point of 64.7 deg. C @1013 hPa (Merck Index, 13th Edition)

Test substance :
Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

Reliability : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a variable mixture

Flag : Critical study for SIDS endpoint
22.11.2003 (8)

2.4 VAPOUR PRESSURE

Value : ca. 90 - 95 hPa at 40 °C

Result : The vapor pressure will vary depending on the exact composition of the mixture. The range given is for the specified mixture. As other compositions of this mixture may be sold, this range may not be universally valid.

In the environment the material will readily dissociate to:

Formaldehyde with a vapor pressure of 5174 hPa @ 25 deg. C (Boublik, T., Fried, V., and Hala, E., The Vapour Pressures of Pure Substances. Second Revised Edition. Amsterdam: Elsevier, 1984. 44 as cited in HSDB)

Methanol with a vapor pressure of 169 hPa @ 25 deg. C (Boublik T et al; The vapor pressures of pure substances: selected values of the temperature dependence of the vapour pressures of some pure substances in the normal and low pressure region. Vol. 17. Amsterdam, Netherlands: Elsevier Sci. Publ 1984. as cited in HSDB)

Test substance :

Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

Reliability : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a variable mixture

Flag : Critical study for SIDS endpoint

22.11.2003 (8)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : ca. -1.4 at 25 °C
pH value :
Method : other (calculated)
Year :
GLP :
Test substance :

Method : Calculated using EPIWIN 3.05 using SMILES input of COCO
Remark :

In the environment the material will readily dissociate to:

Formaldehyde with a log Kow of 0.35 (Hansch, C., Leo, A., D. Hoekman. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society., 1995. 3, as cited in HSDB)

Methanol with a log Kow of -0.77 (ibid.)

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions
EPIWIN calculated values are assigned a reliability of 2.

Flag : Critical study for SIDS endpoint

22.11.2003 (3)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Remark	:	EPIWIN predicted water solubility of pure material is >1000g/L (EPIWIN 3.05 calculation using SMILES of COCO) In the environment the material will readily dissociate to: Formaldehyde with a water solubility >1000g/L (Merck Index, 13th Edition) Methanol with a a water solubility >1000g/L (Merck Index, 13th Edition)
Result	:	Miscible
Test substance	:	Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.
Reliability	:	(2) valid with restrictions Reliability assigned as 2 since this is experimental data on a water reactive mixture.
Flag	:	Critical study for SIDS endpoint

22.11.2003 (8)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Spectrum of substance	:	lambda (max, >295nm) : nm epsilon (max) : epsilon (295) : 0
DIRECT PHOTOLYSIS		
Halflife t1/2	:	> 1 year
Degradation	:	% after
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	cm³/(molecule*sec)
Degradation	:	> 50 % after 15.8 hour(s)
Deg. product	:	
Method	:	other (calculated): APOWIN
Year	:	
GLP	:	
Test substance	:	other TS: Mixture

Method :

As this equilibrium mixture nominally contains methoxymethanol, formaldehyde, methanol and water, and since the initial content of methoxymethanol will be rapidly converted to formaldehyde and methanol, calculations were conducted independently for the three main components.

As there was a discrepancy between the theoretical value of the rate constant for reaction of formaldehyde with hydroxyl radical and an experimental value obtained by Atkinson in 1994, the AOPWIN program was also run on hydrated formaldehyde, which is considered to be in equilibrium with formaldehyde in atmospheres containing water.

Result :

DIRECT PHOTOLYSIS

None of these materials has a chromophore with significant absorption above 295 nm, therefore, direct photolysis is not considered to be an important process in the fate of methoxymethanol preparations.

INDIRECT PHOTOLYSIS

The results of the calculations are shown below. The experimentally derived rate constant for reaction of formaldehyde with hydroxyl radical (Atkinson, 1994) is reconciled by it being a combined rate constant of formaldehyde and hydrated formaldehyde. Formaldehyde is expected to exist in the gas phase as an equilibrium mixture of free and hydrated forms with about a 1:1000 ratio at equimolar concentrations of water. As both the formaldehyde concentration and the atmospheric water concentrations are variables, it is best to assume a range of rate constants and half lives for formaldehyde.

Likewise, methoxymethanol in the vapor phase will react with atmospheric water to produce formaldehyde and methanol. Methanol introduced into the atmosphere, either directly from the mixture or indirectly from hydrolysis of methoxymethanol is considered to exist primarily as the free alcohol in the gas phase when combined with air containing water vapor. The experimentally derived value of the rate constant for the reaction of methanol with hydroxyl radicals is considered more accurate than the predicted value. In addition, as methanol is not as likely to form hydrates, this rate constant is not considered a dependent variable based on atmospheric water content (as is the case with formaldehyde).

Another consideration is polymeric forms of formaldehyde. Due to dilution effects, these are not anticipated to be formed in significant quantity in the vapor phase; however, sublimation of oligomeric formaldehyde from spills of commercial methoxymethanol is possible. The final APOWIN calculation indicates that hydrogen abstraction is very a favorable process for reaction of oligomeric formaldehyde with hydroxyl radical and it will only have an atmospheric half-life on the order of 2 hour. Thus, as it is expected to contribute little to the quantity of material in the air and will not contribute to an extended half-life, it can be ignored relative to atmospheric photodegradation.

In summary, the reaction rate of methoxymethanol or commercial mixtures of formaldehyde, methanol and water with atmospheric hydroxyl radical can be described by the four species listed below.

SPECIES	Half life (12h day 1,500,000 OH molecules/cc)
Methoxymethanol	6.1 hours
Formaldehyde	15.8 hours
Hydroformaldehyde	10.9 hours
Methanol	11.3 hours

As all half-lives are relatively close, the half-life of these mixtures is suffiently well characterized for the purposes of the HPV program as having a range from 6.1 to 15.8 hours

```
Methoxymethanol

AOP Program (v1.90) Results:
============================
SMILES : COCO
CHEM   : Methoxymethanol
MOL FOR: C2 H6 O2
MOL WT : 62.07
-------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS --
Hydrogen Abstraction      =20.7705 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.1400 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =20.9105 E-12 cm3/molecule-sec
 HALF-LIFE =     0.512 Days (12-hr day; 1.5E6 OH/cm3)
 HALF-LIFE =     6.138 Hrs


------------------ SUMMARY (AOP v1.90): OZONE REACTION

         ******  NO OZONE REACTION ESTIMATION ******
         (ONLY Olefins and Acetylenes are Estimated)
```

```
Experimental Database:  NO Structure Matches

############################################################


AOP Program (v1.90) Results:
============================
SMILES : O=C
CHEM   : Formaldehyde
MOL FOR: C1 H2 O1
MOL WT : 30.03
------------ SUMMARY (AOP v1.90): HYDROXYL RADICALS-
Hydrogen Abstraction       =8.1300 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =8.1300 E-12 cm3/molecule-sec
   HALF-LIFE =     1.316 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =    15.787 Hrs

----------------- SUMMARY (AOP v1.90): OZONE REACTION -

         ******  NO OZONE REACTION ESTIMATION ******
          (ONLY Olefins and Acetylenes are Estimated)

Experimental Database Structure Match:
  Chem Name :  Formaldehyde
  CAS Number:  000050-00-0
 Exper OH rate constant  : 9.37  E-12  cm3/molecule-sec
    Exper OH Reference:  KWOK,ESC & ATKINSON,R (1994)
Exper Ozone rate constant: 2.1 E-24  cm3/molecule-sec
Exper NO3 rate constant: 3.2-7.2 E-16  cm3/molecule-sec

_____________________


---------- SUMMARY (AOP v1.90): HYDROXYL RADICALS
SMILES : OCO
CHEM   : HYDRATED FORMALDEHYDE
MOL FOR: C1 H4 O2
MOL WT : 48.04

Hydrogen Abstraction      =11.4415 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.2800 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =11.7215 E-12 cm3/molecule-sec
   HALF-LIFE =     0.913 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =    10.950 Hrs

----------------- SUMMARY (AOP v1.90): OZONE REACTION -

         ******  NO OZONE REACTION ESTIMATION ******
         (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches


####################################################


AOP Program (v1.90) Results:
============================
SMILES : CO
CHEM   : METHANOL
MOL FOR: C1 H4 O1
```

```
MOL WT : 32.04
-------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS -
Hydrogen Abstraction       =0.4760 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.1400 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =0.6160 E-12 cm3/molecule-sec
   HALF-LIFE =    17.364 Days (12-hr day; 1.5E6 OH/cm3)

----------------- SUMMARY (AOP v1.90): OZONE REACTION --

        ******  NO OZONE REACTION ESTIMATION ******
        (ONLY Olefins and Acetylenes are Estimated)

Experimental Database Structure Match:
  Chem Name :  Methanol
  CAS Number:  000067-56-1
Exper OH rate constant :0.944  E-12  cm3/molecule-sec
    Exper OH Reference:  KWOK,ESC & ATKINSON,R (1994)
  Exper Ozone rate constant: --- cm3/molecule-sec
  Exper NO3 rate constant  : --- cm3/molecule-sec

   HALF-LIFE =   11.33 Days (12-hr day; 1.5E6 OH/cm3)

##############################################

SMILES : OCOCOCOCOCOCOCOCO
CHEM   : Polyformaldehyde
MOL FOR: C8 H18 O9
MOL WT : 258.23
-------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS ---
Hydrogen Abstraction      =60.3924 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.2800 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =60.6724 E-12 cm3/molecule-sec
   HALF-LIFE =     0.176 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     2.115 Hrs

---------------- SUMMARY (AOP v1.90): OZONE REACTION ---

          ******  NO OZONE REACTION ESTIMATION ******
          (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches
```

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Conclusion :

All half-lives are relatively close, the half-life of these mixtures has a range from 6.1 to 15.8 hours regarding indirect phtolysis in the atmosphere.

Reliability : (2) valid with restrictions

EPIWIN calculated values are assigned a reliability of 2.

Flag : Critical study for SIDS endpoint

23.11.2003 (4)

3.1.2 STABILITY IN WATER

Type : abiotic
t1/2 pH4 : = 6 minute(s) at 25 °C
t1/2 pH7 : = 6 minute(s) at 25 °C

t1/2 pH9 : = .5 minute(s) at 25 °C
t1/2 pH 2 : = 2 minute(s) at 25 °C
Deg. product : yes
Method : other: chemical kinetics
Year :
GLP :
Test substance :

Method :

The rate of decomposition of methoxymethanol was measured by spectroscopically following the trapping of hydrazine derivatives of formaldehyde hydrolysis product. Determinations were made at different pH levels by recording the change in absorbance against time as a function of pH. These data were used to determine the second order rate constants for hydrolysis of methoxymethanol by water, hydrated protons and hydroxyl ion.

Estimates of hydrolysis rates as a function of pH were made by converting the second order rate constants for the hydrolysis into pseudo first-order rate constants at various pH values and estimating the half-life assuming constant water concentration and pH during the hydrolysis and using the usual relationship between first-order rate constants and half-life.

Result :

The second order rate constants derived for the hydrolysis are:

Reaction with water: k(w) = 3.27 E-5 M^-1 sec^-1
Reaction with H+: k(H) = 0.58
Reaction with OH-: k(OH) = 2.34 E3

Converting these to pseudo-first order rate constants and extrapolation half-lives the following t1/2 are obtained:

	half-life					
Rxn with	2	4	6	7	8	9
Water	6 min	6 min	6 min	6 min	6 min	6 min
Acid	2 min	3.3 hr	333hr	>1000hr	>1000 hr	>1000
Base	>1hr	>1hr	490min	49 min	4.9 min	30 sec

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Conclusion :

Methoxymethanol has a maximum half-life in water of 6 minutes at 25°C. Its pH dependency displays a broad peak from about pH 3 to pH 8. Above or below this range of pH the reaction with acid or base predominates over an already facile reaction with water producing and even shorter half-life. Reaction with base is faster than reaction with acids.

Reliability : (1) valid without restriction

Calculated from peer-reviewed experimental chemical reaction rate constants.

Flag : Critical study for SIDS endpoint
23.12.2003 (7)

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C

Result :

This preparation as typically sold, transported and used is an equilibrium mixture of formaldehyde:methanol:water in a mole ratio of about 3.3:2.0:1.0. The chemical makeup of this mixture is such that there is formally an excess of formaldehyde; however it exists primarily as a series of methanol hemiacetals and hydrates. When added to water, the equilibrium shifts rapidly toward formaldehyde hydrates and methanol.

Methanol is a simple alcohol and alcohols are one of the chemical groups considered stable to hydrolysis (Harris, 1990).

Formaldehyde is known to be water reactive reversibly forming a hydrate (HO-CH2-OH) the equilibrium constant for formaldehyde hydrate formation is > 1000 (Vollhardt, 1987). Thus, formaldehyde is known to be stable indefinitely in water, existing 99.9% as a hydrated species.

Harris, J.C. in Lyman W., Reehl, W. and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington D.C. 1990, page 7-6

Vollhardt, Peter (1987) Organic Chemistry WH Freeman publisher NY p 637

Test substance :

Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

Reliability : (2) valid with restrictions

Estimated values based on sound chemical principles are assigned a reliability of 2.

Flag : Critical study for SIDS endpoint

23.12.2003 (11) (17)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level III
Year :

Method :

Since this mixture contains methoxymethanol, formaldehyde and methanol, and since the initial concentration of methoxymethanol will be readily converted to formaldehyde and methanol the calculations had to be conducted independently.

The actual physical properties for formaldehyde and methanol were input while they were allowed to be calculated for pure methoxymethanol (as they are not known with accuracy). EPIWIN was allowed to set the values for half-lives in various media. Emissions were set to equal values for air

water and soil (the EPIWIN default) for consistency.

SMILES inputs
COCO
CO
C=O

Result :

The calculations indicate that all three major components distribute primarily to water followed closely by soil. Only methanol indicates that it we distribute to air more than a few percent. As this is a variable mixture in actual production and use, and as these materials have high water solubility and biodegradability these estimates are adequate to understand the approximate distribution of the material in the environment.

```
Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : Methoxymethanol
  Molecular Wt: 62.07
  Henry's LC  : 1.47e-006 atm-m3/mole (Henrywin program)
  Vapor Press : 32 mm Hg  (Mpbpwin program)
  Log Kow     : -1.4  (Kowwin program)
  Soil Koc    : 0.0163  (calc by model)

         Concentrat Half-Life Emissions
           (percen)   (hr)     (kg/hr)
   Air       1.92      12.3      1000
   Water     54.8       360      1000
   Soil      43.2       360      1000
   Sediment  0.0913    1440        0

            Fugacity  React  Advect Reaction  Advection
             (atm)    kg/h) (kg/h)  (percent) (percent)
   Air       6.12e-011  878    155     29.3      5.18
   Water     5.24e-011  852    442     28.4      14.7
   Soil      1.53e-009  672     0      22.4      0
   Sediment  4.36e-011  .355   .0147   .0118     .000492

   Persistence Time: 269 hr
   Reaction Time:    336 hr
   Advection Time:   1.35e+003 hr
   Percent Reacted:  80.1
   Percent Advected: 19.9

   Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
      Air:      12.28
      Water:    360
      Soil:     360
      Sediment: 1440
        Biowin estimate: 3.213  (weeks)

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004



METHANOL

Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : methanol
  Molecular Wt: 32.04
  Henry's LC  : 4.55e-006 atm-m3/mole (Henry database)
  Vapor Press : 127 mm Hg  (user-entered)
  Log Kow     : -0.77  (Kowwin program)
  Soil Koc    : 0.0696  (calc by model)

         Concentration   Half-Life    Emissions
           (percent)        (hr)       (kg/hr)
   Air       13             272          1000
   Water     47.2           208          1000
   Soil      39.7           208          1000
   Sediment  0.0705         832          0
```

```
                Fugacity  Reaction  Advection  Reaction  Advection
                 (atm)    (kg/hr)   (kg/hr)   (percent)  (percent)
   Air       5.96e-010  199       782        6.64      26.1
   Water     2.01e-010  943       283        31.4      9.44
   Soil      6.22e-009  792       0          26.4      0
   Sediment  1.5e-010   .352      .00846     0.0117    0.000282

   Persistence Time: 200 hr
   Reaction Time:    310 hr
   Advection Time:   563 hr
   Percent Reacted:  64.5
   Percent Advected: 35.5

   Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
      Air:      271.9
      Water:    208.1
      Soil:     208.1
      Sediment: 832.3
        Biowin estimate: 3.288  (days-weeks  )

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004
```

```
FORMALDEHYDE

Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : Formaldehyde
  Molecular Wt: 30.03
  Henry's LC  : 3.37e-007 atm-m3/mole (Henry database)
  Vapor Press : 3.89e+003 mm Hg  (user-entered)
  Liquid VP   : 2.04e+004 mm Hg  (super-cooled)
  Melting Pt  : 97.8 deg C (user-entered)
  Log Kow     : 0.35  (user-entered)
  Soil Koc    : 0.918  (calc by model)

          Concentration   Half-Life    Emissions
            (percent)        (hr)       (kg/hr)
   Air       2.7             27.4         1000
   Water     51.3            360          1000
   Soil      45.9            360          1000
   Sediment  0.0871          1.44e+003    0

             Fugacity Reaction Advection Reaction  Advection
              (atm)   (kg/hr)   (kg/hr)  (percent) (percent)
   Air       1.98e-010  614      243       20.5      8.09
   Water     2.59e-011  887      461       29.6      15.4
   Soil      7.99e-010  795      0         26.5      0
   Sediment  2.15e-011  0.377    0.0157    0.0126    0.000522

   Persistence Time: 300 hr
   Reaction Time:    391 hr
   Advection Time:   1.28e+003 hr
   Percent Reacted:  76.5
   Percent Advected: 23.5

   Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
      Air:      27.41
      Water:    360
      Soil:     360
      Sediment: 1440
        Biowin estimate: 3.155  (weeks)

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004
```

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions

EPIWIN calculated values are assigned a reliability of 2.

Flag : Critical study for SIDS endpoint

22.11.2003 (6)

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	other: not pre-acclimated inoculum
Contact time	:	
Degradation	:	= 90 (±) % after 28 day(s)
Result	:	readily biodegradable
Deg. product	:	
Method	:	OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
Year	:	1990
GLP	:	no
Test substance	:	other TS
Remark	:	Result adopted from SIDS 2003 document. Material was agreed to be readily biodegradable at the SIAM meeting
Test substance	:	Formaldehyde CASNO 50-00-0
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

22.11.2003 (10)

Type	:	aerobic
Inoculum	:	activated sludge, domestic, non-adapted
Contact time	:	
Degradation	:	= 50 - 80 (±) % after 6 day(s)
Result	:	
Remark	:	This robust summary was adopted from the Methanol HPV document. Please see the Methanol HPV document for additional studies. Methanol has been well studied in biodegradation assays of several types and the weight of evidence indicates it is readily biodegradable.
Test substance	:	Methanol
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

22.11.2003 (15)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	other: ECOSAR Estimate
Species	:	other: freshwater fish
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	ca. 45 calculated
Method	:	other: Estimate
Year	:	
GLP	:	
Test substance	:	

Method :

The SMILES formula for methoxymethanol (COCO) was entered into ECOSAR (via EPIWIN 3.05). The program calculated critical physical properties and applies them to the neutral organic model to estimate the LC50 for fish. This was further evaluated for reasonableness and it was determined to be reasonable on chemical grounds. It was recognized, however, that hydrolysis of methoxymethanol will produce formaldehyde, which is a reactive chemical that will not fit the neutral organics model.

Remark :

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to fish (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to fish, formaldehyde is the species that will determine the acute toxicity of this mixture to fish. In recognition of this, the robust summary flagged as "critical for SIDS endpoint" has been adopted from the formaldehyde SIDS document. The reader is referred to the formaldehyde SIDS document for more supporting studies.

The critical study was amongst the lowest of the LC50 values, and while it is recognized that there is a possibility that there will synergetic interactions between formaldehyde, this predicted LC50 is considered conservative as it was from a highly sensitive species. Significant synergism between formaldehyde and methanol is considered unlikely, as formaldehyde is a metabolic product of methanol and methanol will not distribute strongly into fish tissues due to its Kow.

Result :

The ECOSAR estimate (in its entirety) is presented for completeness but the LC50 for methoxymethanol is estimated at 55% (the weight percent of formaldehyde in the mixture) of the published LC50 for formaldehyde.

```
ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
                                                        Predicted
ECOSAR Class          Organism    Duration End Pt   mg/L
==================== =========== ======== ====   ==========
Neutral Organic SAR: Fish          14-day  LC50  76256.125
(Baseline Toxicity)

Neutral Organics   : Fish          96-hr   LC50  72256.133
Neutral Organics   : Fish          14-day  LC50  76256.125
Neutral Organics   : Daphnid       48-hr   LC50  61217.387
Neutral Organics   : Green Algae   96-hr   EC50  31468.400
Neutral Organics   : Fish          30-day  ChV    5381.146
Neutral Organics   : Daphnid       16-day  EC50    709.373
Neutral Organics   : Green Algae   96-hr   ChV     441.037
Neutral Organics   : Fish  (SW)    96-hr   LC50   3197.973
Neutral Organics   : Mysid Shrimp  96-hr   LC50   2.36e+005
Neutral Organics   : Earthworm     14-day  LC50   4256.716
```

Estimate based on formaldehyde toxicity 1/55% of 24.8 = 45 mg/L for freshwater fish.

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions

Based on toxic component. Considered an acceptable scientific method to conduct estimate

Flag : Critical study for SIDS endpoint

23.11.2003 (5)

Type : flow through
Species : Ictalurus melas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 24.8 measured/nominal
Limit test :
Analytical monitoring : no
Method : other: acute toxicity test; "flow through bioassay"
Year : 1977
GLP : no
Test substance : other TS: formalin, commercial grade, 37%

Method :

fingerling; pH 6.5, water hardness 8, water temperature 12 degrees Centigrade

Remark :

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to fish (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to fish, formaldehyde is the species that will determine the acute toxicity of this mixture to fish. In recognition of this, the robust summary flagged as "critical for SIDS endpoint" has been adopted from the formaldehyde SIDS document. The reader is referred to the formaldehyde SIDS document for more supporting studies.

Result :

Test result: 62.1 µl/l formalin (solution 37%)

Test substance :

Formaldehyde CASNO 50-00-0

Reliability : (2) valid with restrictions

Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

23.11.2003 (1)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	other: estimate
Species	:	other: freshwater invertebrate
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	ca. 10.5 calculated
Method	:	other: Estimate
Year	:	
GLP	:	
Test substance	:	

Method :

The SMILES formula for methoxymethanol (COCO) was entered into ECOSAR (via EPIWIN 3.05). The program calculated critical physical properties and applies them to the neutral organic model to estimate the EC50 for daphnia. This was further evaluated for reasonableness and it was determined to be reasonable on chemical grounds. It was recognized, however, that hydrolysis of methoxymethanol will produce formaldehyde, which is a reactive chemical that will not fit the neutral organics model.

Remark :

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to invertebrates (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to daphnids, formaldehyde is the species that will determine the acute toxicity of this mixture to invertebrates. In recognition of this, the robust summary flagged as "critical for SIDS endpoint" has been adopted from the formaldehyde SIDS document. The reader is referred to the formaldehyde SIDS document for more supporting studies.

The critical study was amongst the lowest of the EC50 values, and while it is recognized that there is a possibility that there will synergetic interactions between formaldehyde, this predicted EC50 is considered conservative as it was from a highly sensitive species. Significant synergism between formaldehyde and methanol is considered unlikely, as formaldehyde is a metabolic product of methanol and methanol will not distribute strongly into invertebrates tissues due to its Kow.

Result :

The ECOSAR estimate (in its entirety) is presented for completeness but the EC50 for methoxymethanol is estimated at 1/55% (the weight percent of formaldehyde in the mixture) of the published EC50 for formaldehyde.

```
ECOSAR v0.99f Class(es) Found
```

```
-----------------------------
Neutral Organics
                                                    Predicted
ECOSAR Class          Organism   Duration End Pt   mg/L
==================== =========== ======= ====   ==========
Neutral Organic SAR: Fish         14-day  LC50   76256.125
(Baseline Toxicity)

Neutral Organics   : Fish         96-hr   LC50   72256.133
Neutral Organics   : Fish         14-day  LC50   76256.125
Neutral Organics   : Daphnid      48-hr   LC50   61217.387
Neutral Organics   : Green Algae  96-hr   EC50   31468.400
Neutral Organics   : Fish         30-day  ChV     5381.146
Neutral Organics   : Daphnid      16-day  EC50     709.373
Neutral Organics   : Green Algae  96-hr   ChV      441.037
Neutral Organics   : Fish  (SW)   96-hr   LC50    3197.973
Neutral Organics   : Mysid Shrimp 96-hr   LC50    2.36e+005
Neutral Organics   : Earthworm    14-day  LC50    4256.716

Estimate based on formaldehyde toxicity 1/55% of 5.8  = 10.5 mg/L for
daphnids.
```

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions

Based on toxic component. Considered an acceptable scientific method to conduct estimate

Flag : Critical study for SIDS endpoint

23.11.2003 (5)

Type : other: According to OECD standard
Species : Daphnia pulex (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC50 : = 5.8 measured/nominal
EC10 : = measured/nominal
EC90 : = 16.8 measured/nominal
Limit Test : no
Analytical monitoring : no data
Method :
Year :
GLP : no data
Test substance : other TS: Formaldehyde

Result :

EC50 (48 h) = 4.3 - 7.8 (confidence limit)

Test condition :

test temperature 20 +/- 1 °C,
the standard stock solutions were prepared according to Standard Methods: APHA-AWWA-WEF, 1992 and Leithe, 1974, daphnids cultured in 3-L-aquariumsand beakers were illuminated 12 hr per day

Test substance :

Formaldehyde 37 % v/v

Reliability : (2) valid with restrictions

accepatable study, meets basic scientific principles

23.11.2003 (16)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	other algae: green generic
Endpoint	:	biomass
Exposure period	:	72 hour(s)
Unit	:	mg/l
EC10	:	= calculated
Method	:	other: Estimation
Year	:	2003
GLP	:	
Test substance	:	

Remark :

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to algae (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to algae, formaldehyde is the species that will determine the acute toxicity of this mixture to aquatic plants. In recognition of this, two robust summaries flagged as "critical for SIDS endpoint" have been adopted from the formaldehyde SIDS document for use in the estimation of methoxymethanol toxicity to aquatic plants. The reader is referred to the formaldehyde SIDS document for additional supporting studies.

The critical studies were a long duration (192 hour) and a short duration (24 hour) study using the same species. Different endpoints were used and the estimated toxicity of methoxymethanol was calculated by taking the geometric mean of the toxic threshold value from the 192-hour study and the EC50 of the 24-hour study and setting this as the 72-hour EC50 for formaldehyde. Although there is no known scientific precedent for this calculation, it recognizes that the true value of the 72-hour EC50 for formaldehyde is lower than the 24-hour EC50. It is also recognized that formaldehyde undoubtedly reacted with the algae reducing its concentration greatly in the 192-hour study and probably in the 24-hour study. These data are considered acceptable for the estimate as ODED has recently accepted this data set for formaldehyde and as it would be impossible to accurately determine the EC50 of formaldehyde due to its reactivity and volatility.

Result :

The SMILES formula for methoxymethanol (COCO) was entered into ECOSAR (via EPIWIN 3.05). The program calculated critical physical properties and applies them to the neutral organic model to estimate the EC50 for algae. This was further evaluated for reasonableness and it was determined to be reasonable on chemical grounds. It was recognized, however, that hydrolysis of methoxymethanol will produce formaldehyde, which is a reactive chemical that will not fit the neutral organics model.

The ECOSAR estimate (in its entirety) is presented for completeness but the EC50 for methoxymethanol is estimated at 1/55% (the weight percent of formaldehyde in the mixture) of the calculated EC50 for formaldehyde.

```
ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
                                                        Predicted
ECOSAR Class          Organism    Duration End Pt   mg/L
==================== =========== ======= ====   ==========
Neutral Organic SAR: Fish          14-day  LC50  76256.125
(Baseline Toxicity)

Neutral Organics   : Fish          96-hr   LC50  72256.133
Neutral Organics   : Fish          14-day  LC50  76256.125
Neutral Organics   : Daphnid       48-hr   LC50  61217.387
Neutral Organics   : Green Algae   96-hr   EC50  31468.400
Neutral Organics   : Fish          30-day  ChV    5381.146
Neutral Organics   : Daphnid       16-day  EC50    709.373
Neutral Organics   : Green Algae   96-hr   ChV     441.037
Neutral Organics   : Fish  (SW)    96-hr   LC50   3197.973
Neutral Organics   : Mysid Shrimp  96-hr   LC50   2.36e+005
Neutral Organics   : Earthworm     14-day  LC50   4256.716

Estimate based on formaldehyde toxicity 1/55% of 6.0 (geometric mean
of 196-hour EC03 and 24-hour EC50)   = 11.5 mg/L for green algae.
```

Test substance :

Methoxymethanol CASNO 4461-52-3, assumed pure

Reliability : (2) valid with restrictions

Based on toxic component. Considered an acceptable scientific method to conduct estimate

Flag : Critical study for SIDS endpoint

23.11.2003 (5)

Species : Scenedesmus quadricauda (Algae)
Endpoint : biomass
Exposure period : 192 hour(s)
Unit : mg/l
EC03 : = .88 measured/nominal
Method : other: Static Cell Multiplication Inhibition Test
Year : 1978
GLP : no
Test substance : other TS: Formaldehyde

Result :

Toxicity Threshold : 2.5 mg/l 35% formalin
0.88 mg/l Formaldehyde

Toxic threshold is defined in this investigation as the concentration of test substance causing 3 % inhibition of cell multiplication compared to untreated controls.

Test condition :

Test vessel: Kapsenberg cultivation tubes (18 x 180mm)

Concentration of stock solution: not indicated

Pre-treatment of test solution: neutralisation if necessary

Inoculum:cell density adjusted to TE/F = 20 formazin turbidity equivalents at 578nm)

Test volume: 10 ml

Dilution:1:2
Number of test replicates:3

Number of control replicates:1

Illumination:constant artifical light (Osram L 40/30)

Temperature: 27 °C

Agitation: once daily

Measurements:photometric determination of cell density at578 nm after 192 h of exposure

Test substance :

Formalin (35% solution)

Reliability : (2) valid with restrictions

Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

23.11.2003 (2)

Species : Scenedesmus quadricauda (Algae)
Endpoint : other: Oxygen uptake and produstion
Exposure period : 24 hour(s)
Unit : mg/l
EC10 : = 3.6 measured/nominal
EC50 : = 14.7 measured/nominal
EC90 : = 60.3 measured/nominal
Method :
Year :
GLP : no data
Test substance : other TS: Formaldehyde

Method :

Toxicity to algae was evaluated by measuring the oxygen production and consumption rates following exposure to the test media and calculating the 24-hr net assimilation by the algae.

The oxygen production and consumption rates were measured on Warburg apparatus (type 85G, B.Braun, Germany)

The effective concentrations were calculated using linear regression analysis.

Remark :

Short duration

Test condition :

test temperature 20 +/- 1 °C,
Standard stock solutions were prepared according to Standard Methods: APHA-AWWA-WEF, 1992 and Leithe, 1974,cultured in the nutrient solution prepared according to Holm Hansen (Bringmann and Kühn, 1980) under continuous illumination (3000 lx)

Test substance :

Formaldehyde (37% solution in water)

Reliability : (2) valid with restrictions

accepatable study, meets basic scientific principles

23.11.2003 (16)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 1269 mg/kg bw
Species	:	rat
Strain	:	Crj: CD(SD)
Sex	:	male/female
Number of animals	:	
Vehicle	:	water
Doses	:	707, 1000, 1414, 2000, 2828
Method	:	
Year	:	
GLP	:	yes
Test substance	:	

Method :

Specific guideline not specified.

The test substance in distilled water (dissolved just before administration) was administered by gavage to groups of five rats of each sex that had been fasted overnight. Doses, based on a range-finding study, were 707, 1000, 1414, 2000, and 2828 mg/kg. The volume of administration was 10 ml/kg and feed was not given for approximately three hours after administration. Purity was not determined when the test solution was prepared.

General conditions of animals were observed on the day of administration at 5 minutes, 15 minutes, 30 minutes, 1 hour, 3 and 6 hours after dosing, and once a day for a period of 14 days. Body weight was measured just before treatment, and on days 3, 7 and 14. Dead animals were necropsied promptly after discovery. After the 14-day observation period, surviving animals were sacrificed and examined. The LD50 was computed using the probit method.

Result :

Mortality was observed as indicated in the table below:

Dose	MORTALITY	
	Males	Females
0	0/5	0/5
707	0/5	0/5
1000	1/5	1/5
1414	3/5	2/5
2000	5/5	4/5
2828	5/5	5/5

Most deaths were within an hour of administration

LD50 values were 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg), and 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg)

Clinical Observations: Reduced spontaneous activity, slow breathing and blepharoptosis were observed across all groups; groups at 2000 mg/kg and

above showed lying down, gasping and clonic seizures. Salivation was reported among all groups other than the female 707 and 1414 mg/kg groups. Other symptoms included lacrimation, red lacrimation, red nasal drainage and raising of the tail.

Body Weights: Some of the surviving animals in 1414 mg/kg and 2000 mg/kg groups showed weight loss on the third day post-administration, but gained weight thereafter. Surviving animals of the other groups gained weight throughout the period of observation.

Necropsy: Animals dying from treatment showed atrial enlargement, pulmonary congestion/edema, and congestion/ edema/hemorrhaging/erosion of glandular stomach mucous membrane. Among surviving animals, adhesions of stomach and liver, thickening of the anterior stomach mucous membrane and erosion/ulceration of glandular stomach mucus membrane were noted in the groups at dose levels of 2000 and 2828 mg/kg.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Conclusion :

The LD50 values were:

Males: 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg)
Females: 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg)

No specific target organs were identified.

Reliability : (1) valid without restriction
Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

21.08.2003 (14)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	41 to 47 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	12, 60 or 300 mg/kg-day
Control group	:	yes, concurrent vehicle

Method : other: OECD Guideline 422
Year :
GLP : yes
Test substance :

Method :

Sprague-Dawley rats (Crj:CD, SPF) obtained from Charles River Laboratories, Japan were acclimated for six days before they were divided into groups of 10 animals of each sex using stratified random sampling by weight. Rats were 8 weeks old and their weight ranged from 278-309g for males and 186-215g for females at the first dosing.

The animal room used a 12-hour day light cycle and was regulated to maintain the temperature between 20-25° C, the humidity between 40-70% R.H., and ventilation at about 12 changes of air per hour. Animals were housed in polycarbonate boxes using bedding (Betachip: Charles River Laboratories, Japan). Except during breeding, when one male and one female were co-housed, animals were individually housed. After delivery, the dam and her litter were kept in the same cage during the lactation period.

Autoclaved feed (CRF-1: Oriental Yeast Co., Ltd.) and tap water that was filtered through a 5μm filter and was irradiated with ultraviolet rays were offered ad lib.

DOSE SELECTION: Dose levels of 0,12, 60 or 300 mg/kg-day were selected based on a preliminary study with dose levels of 0, 30, 100, 300 or 1000 mg/kg-day. The 1000 mg/kg-day group showed signs of overt toxicity including reduced spontaneous activity, irregular respiration, lacrimation and death. Necropsy revealed erosion or ulceration of the stomach or duodenum in the high-dose group. The 300 mg/kg-day group was reported to show salivation and changes in the stomach but these effects were considered a LOAEL and 300 mg/kg-day was selected as the high dose for the definitive study.

STUDY CONDUCT: Males were dosed for 44 days starting 14 days prior to mating and were sacrificed the day after the last dosing. Females were dosed for 41 to 47 days starting 14 days before mating, through mating and delivery, and three days of lactation. The test substance was diluted with distilled water prior to dosing and given by gavage as a single daily administration in the morning. Dosing volume was 5ml/kg calculated based on the most current body weight measured at that time.

Rats were mated one male and one female within the same group and allowed to mate for seven days. During this period, every day in the morning, the female's vaginal mucus was collected and was microscopically examined after it was Giemsa stained. Day zero of gestation was recorded when either a vaginal plug or sperm was found in the vaginal specimen.

Pregnant females were allowed to deliver their pups naturally. Lactation day zero was defined as completion of delivery by 9:00 in the morning of day zero. Pups were allowed to nurse until lactation day 4 and observed daily during this time for general condition, lactation, nesting, cannibalism and other significant signs. Surviving dams and pups were sacrificed on lactation-day 4. Ovaries and uteri of dams were removed to count corpora lutea and implantation sites. Based on the results obtained from these examinations, the gestation period, the gestation index, the implantation index and the delivery index were calculated.

EXAMINATION OF PUPS: Dead pups, except those that were killed and eaten and unfit for examination, were fixed in a mixed solution of formaldehyde and acetic acid before being microscopically examined. Pups from each dam were separated by sex and weighed as a group of one sex on days zero and 4. External examinations, including the oral cavity, were conducted on lactation day 4. After the examination, about half of the pups from each litter were sacrificed and prepared for skeletal examination. Pups from the control group and the high-dose group were examined for skeletal abnormalities. Pups not selected for skeletal examination were submitted to visceral examinations after fixation with a mixture of formaldehyde and acetic acid. Heads from the control and high-dose groups were examined using Wilson's method and their chest and abdomen were micro-dissected to discover any visceral abnormalities. Since there was a slightly increased occurrence of patent foramen ovale in the 300 mg/kg-day group, the 60 mg/kg-day group was also examined for visceral abnormalities.

STATISTICAL METHODS: Data were tested for homogeneity using Bartlett's method and when the distribution was normal, a one-way distribution dispersion analysis was performed. Then using either Dunnett's or Scheffe's test, the mean values were compared. When the distribution was not normal, the Kruskal-Wallis test was applied before the rank sum test of either Dunnett's or Scheffe's method. Some parameters (with asterisk) were tested initially using the Kruskal-Wallis test and when there was a significant difference, the rank sum test was performed. The calculated data were tested using Fisher's direct probability method. The level of significance was set to 5%. The mean values calculated from each maternal group were used as their statistical units for the data pertaining to the newborn pups. The following are the items for the statistical analysis.

Multiple comparison tests were used with: Weight, weight gain, feed consumption, hematological tests, blood biochemistry tests, weight of organs, paring days*, number of estrous cycles before successful copulation*, gestation period*, number of corpora lutea, number of implantation sites, implantation index*, delivery index*, number of newborn pups, weight of newborn pups, live birth index*, viability index*, and the occurrence of skeletal and visceral abnormalities among live pups*

Fisher's direct probability method was used with: Copulation index, fertility index, gestation index, and sex ratio (male/female)

Result :

DEATHS: One male from the 300 mg/kg-day group died on the 14th day of administration.

CLINICAL SIGNS: Slight salivation after administration of the test substance was observed in the 300 mg/kg-day group starting on the second administration day for males, and the fourth day for the females lasting and was observed for almost all animals. Some started salivating even before the dose was given and one male showed decreased spontaneous activities and gasping on the 13th day before dying the next day. One female was observed with rales starting on the 12th day of administration and lasting through the 6th day of gestation. A few males and females in the 60 mg/kg-day group also displayed salivation but this was a sporadic occurrence.

BODY WEIGHTS: Suppression of body weight gain was noted among males of the 300 mg/kg-day group from the 7th day of administration throughout the rest of the administration period. Females did not show any significant

difference between controls and dosed groups throughout the periods before mating, during gestation and after delivery.

FEED CONSUMPTION: Reduced feed consumption was noted for high dose males starting on the seventh day of dosing and continuing until sacrifice. Feed consumption for other dose groups was not different from controls before mating, during gestation period and after delivery.

HEMATOLOGY: A decrease in the red blood cell count, hematocrit value and hemoglobin concentration was noted for the high dose males as well as an increase in both reticulocyte and platelet counts. The leukocyte differential count was unremarkable for all dosed groups.

BIOCHEMISTRY: A decrease in the total protein, albumin and calcium and an increase in the A/G ratio were noted in the high-dose males. Chloride was also increased in the high-dose males but the increase was very slight and is not considered toxicologically significant.

ORGAN WEIGHTS: There was no significant difference in any of the organs between the control group and the dosed groups.

GROSS EXAMINATION: Either ulceration or erosion of the gastric glands and the proventriculus mucus membrane of the stomach were noted in 3 males and 2 females in the 300 mg/kg-day group. Five males and 4 females in the high-dose group showed the formation of gastric nodules in various sizes. Six high-dose males showed an enlarged duodenum. One high-dose male showed enlarged adrenal glands. The high-dose male that died on test had an enlarged atrium, pulmonary congestion, atrophy of the thymus gland, red patches in the gastric gland mucosa and distension of the bowel.

MICROSCOPIC EXAMINATION: Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300 mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border.

Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls.

Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Attached document :

Organ Wts.bmp
Hematol-ps.bmp
Biochem-ps2.bmp
Histopath.bmp

Table 3 Absolute and relative organ weight of rats treated orally with methoxymethanol in combined repeat dose and reproductive/ developmental toxicity screening test

Sex	Dose level	0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
Male					
	No of animals	10	10	10	9
	Body weight (g)	445 ± 26.1	440 ± 25.8	449 ± 32.5	393 ± 45.0*
	Absolute organ weight				
	Thymus (mg)	358 ± 60.0	415 ± 80.7	335 ± 44.3	287 ± 90.9
	Liver (g)	12.27 ± 1.547	11.89 ± 1.397	12.21 ± 1.373	11.45 ± 1.195
	Kidneys (g)	2.86 ± 0.250	2.93 ± 0.305	2.82 ± 0.236	2.72 ± 0.269
	Testes (g)	3.39 ± 0.227	3.15 ± 0.147	3.21 ± 0.344	3.27 ± 0.277
	Epididymides (g)	1.26 ± 0.121	1.22 ± 0.087	1.25 ± 0.127	1.23 ± 0.120
	Relative organ weight				
	Thymus (mg%)	80 ± 10.7	95 ± 19.0	75 ± 9.0	72 ± 19.3
	Liver (g%)	2.75 ± 0.213	2.70 ± 0.209	2.71 ± 0.133	2.92 ± 0.233
	Kidneys (g%)	0.64 ± 0.028	0.67 ± 0.057	0.63 ± 0.041	0.69 ± 0.046
	Testes (g%)	0.75 ± 0.081	0.72 ± 0.056	0.72 ± 0.049	0.84 ± 0.112
	Epididymides (g%)	0.28 ± 0.025	0.28 ± 0.024	0.28 ± 0.018	0.32 ± 0.026
Female					
	No of animals	10	10	10	9
	Body weight (g)	313 ± 14.8	315 ± 22.4	312 ± 19.7	310 ± 14.8
	Absolute organ weight				
	Thymus (mg)	199 ± 69.2	216 ± 71.0	236 ± 101.8	185 ± 31.9
	Liver (g)	14.25 ± 0.945	13.84 ± 1.876	13.83 ± 0.567	15.10 ± 1.477
	Kidneys (g)	2.10 ± 0.255	2.11 ± 0.249	2.29 ± 0.574	2.01 ± 0.130
	Relative organ weight				
	Thymus (mg%)	63 ± 21.2	69 ± 24.2	75 ± 29.0	60 ± 9.6
	Liver (g%)	4.55 ± 0.255	4.38 ± 0.370	4.45 ± 0.320	4.87 ± 0.446
	Kidneys (g%)	0.67 ± 0.037	0.67 ± 0.067	0.71 ± 0.176	0.65 ± 0.041

Values are expressed as Mean ± S.D.
Significantly different from control group; *: P<0.05.

Table 1 Hematology of male rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test.

Dose level	0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
No. of aminals	10	10	10	9
RBC ($\times 10^4$/mm^3)	813 ± 6.0	815 ± 41.7	820 ± 24.7	755 ± 52.0*
Hematocrit (%)	43.7 ± 1.00	43.6 ± 1.16	43.6 ± 1.18	38.7 ± 4.66**
Hemoglobin (g/dl)	15.5 ± 0.41	15.4 ± 0.53	15.6 ± 0.32	13.5 ± 1.92**
Reticulocyte (‰)	25 ± 3.5	26 ± 4.4	26 ± 2.8	45 ± 18.7**
MCV (μm^3)	53.8 ± 1.33	53.6 ± 1.88	53.1 ± 1.62	51.2 ± 3.92
MCH (pg)	19.1 ± 0.60	19.0 ± 0.71	19.0 ± 0.38	17.8 ± 1.81
MCHC (%)	35.5 ± 0.43	35.4 ± 0.48	35.7 ± 0.53	34.7 ± 1.07
Platelet ($\times 10^4$/mm^3)	102.8 ± 11.02	103.3 ± 13.55	106.6 ± 17.65	127.4 ± 30.09**
WBC ($\times 10^2$/mm^3)	104 ± 31.4	107 ± 29.8	104 ± 20.8	103 ± 33.4
Differential leukocyte counts (%)				
Lymphocytes	78 ± 8.6	81 ± 6.2	83 ± 6.0	76 ± 8.5
Neutrophils				
segmented	16 ± 7.8	12 ± 5.2	11 ± 6.0	19 ± 6.2
band	0 ± 0.3	1 ± 0.9	1 ± 0.8	1 ± 0.5
Eosinophils	1 ± 0.5	1 ± 0.9	1 ± 1.2	1 ± 0.7
Basophils	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Monocytes	5 ± 1.9	5 ± 1.6	4 ± 2.0	4 ± 4.1

Values are expressed as Mean ± S.D.
Significantly different from control group; *: P<0.05, **: P<0.01.

Table 2 Blood chemistry of male rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

Dose level	0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
No. of aminals	10	10	10	9
GOT (IU/l)	83 ± 14.1	84 ± 13.1	78 ± 11.6	93 ± 11.8
GPT (IU/l)	27 ± 5.6	26 ± 3.5	26 ± 4.0	33 ± 9.7
γ-GTP (IU/l)	0 ± 0.0	0.1 ± 0.316	0 ± 0.0	0 ± 0.0
ALP (IU/l)	283 ± 32.6	245 ± 54.3	233 ± 50.9	199 ± 53.8
Total bilirubin (mg/dl)	0.11 ± 0.032	0.05 ± 0.053**	0.10 ± 0.00	0.09 ± 0.033
Urea nitrogen (mg/dl)	18.5 ± 2.08	18.8 ± 2.64	18.7 ± 2.58	17.1 ± 4.06
Creatinie (mg/dl)	0.5 ± 0.03	0.5 ± 0.03	0.5 ± 0.06	0.4 ± 0.05
Glucose (mg/dl)	126 ± 8.1	128 ± 13.3	132 ± 13.7	115 ± 23.8
Total chol. (mg/dl)	75 ± 21.8	65 ± 14.8	69 ± 11.0	69 ± 8.9
Triglyceride (g/dl)	58 ± 28.4	49 ± 20.8	74 ± 36.1	64 ± 25.0
Total protein (g/dl)	6.69 ± 0.187	6.33 ± 0.476	6.46 ± 0.260	5.61 ± 0.312**
Albumin (g/dl)	3.71 ± 0.083	3.61 ± 0.229	3.70 ± 0.110	3.38 ± 0.157**
A/G ratio	1.25 ± 0.050	1.33 ± 0.074	1.34 ± 0.058	1.53 ± 0.190**
Ca (mg/dl)	9.4 ± 0.22	9.3 ± 0.32	9.3 ± 0.21	8.9 ± 0.17**
Inorganic phos. (mg/dl)	7.4 ± 0.46	7.6 ± 0.37	7.5 ± 0.45	7.5 ± 0.66
Na (meq/l)	144 ± 0.6	144 ± 1.0	144 ± 0.9	144 ± 0.8
K (meq/l)	4.5 ± 0.17	4.5 ± 0.25	4.5 ± 0.10	4.6 ± 0.52
Cl (meq/l)	105 ± 1.3	106 ± 2.0	105 ± 1.0	107 ± 1.3**

Values are expressed as Mean ± S.D.
Significantly different from control group; **: P<0.01.

Table 5 Summary of histopathological findings in rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

Organ	Sex:	Male				Female			
	Dose level (mg/kg):	0	12	60	300	0	12	60	300
findings	No. of animals:	10	10	10	9	10	10	10	10
Stomach									
Ulcer		0	0	0	5	0	0	0	8
Erosion		0	0	2	3	0	0	0	2
Focal regenerative change of gastric gland		0	0	3	6	0	0	0	5
Inflammatory cell infiltration in submucosal layer		0	0	0	9	0	0	0	5
Duodenum									
Thickening of mucosa		0	0	0	6	0	$	$	0
Adrenals									
Hypertrophy of zona fasciculata and zona reticularis		0	0	0	2	0	0	0	0
Kidenys									
Basophilic change of the tubular epithelium		0	$	$	0	2	$	1/1 #)	0
Liver									
Peripheral fatty change		0	$	1/1	0	0	$	$	0
Focal necrosis		0	$	$	1	0	$	$	0
Skin									
Erosion		$	1/1	$	$	$	$	$	$

$: Not examined, #:Number of animals with lesion / Number of animals examined.

Conclusion :

Toxic effects related to administration of the test substance were observed primarily in the digestive tract and are considered to result primarily from the irritating property of the test substance. For males effects were seen at 60 mg/kg-day and above. For females, effects were seen only at the high dose.

Regarding hematology, changes in RBC's (reduced number), reticulocytes and platelets (increased) were only seen in the high-dose males. These effects may have been related to gastric ulceration and subsequent loss of blood.

Regarding clinical chemistry, effects were found only for the high-dose males. The reduction in total protein and albumin and the albumin/globulin ratio are also consistent with gastric ulceration and subsequent loss of blood.

Effects appear to be primarily at the site of contact and related to the irritant properties of the test substance. The GI tract is identified as the target organ and biochemical and hematologic changes are considered secondary to gastric ulceration and subsequent loss of blood.

The following effect levels are assigned:
LOAEL
60 mg/kg-day (males)
300 mg/kg-day (females)

NOAEL
12 mg/kg-day (males)
60 mg/kg-day (females)

Reliability : (1) valid without restriction

Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint
01.12.2003 (9)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Bacterial reverse mutation assay
System of testing	:	Salmonella typhimurium TA100, TA1535, TA98, TA 1537 and E coli WP2 uvrA
Test concentration	:	Up to 625 micrograms/plate for Salmonella and 2500 micrograms/plate for E coli.
Cycotoxic concentr.	:	Salmonella 500 micrograms/plate and above E coli 2500 micrograms/plate and above
Metabolic activation	:	with and without
Result	:	positive
Method	:	
Year	:	
GLP	:	no data
Test substance	:	

Method :

Using the plate incorporation method, the following bacterial strains were exposed to test material in the presence and absence of S9 mix (prepared from Sprague-Dawley type male rats induced by concurrent administration of phenobarbital and 5, 6-benzoflavone):

Salmonella typhimurium TA100
Salmonella typhimurium TA1535
Escherichia coli WP2 uvrA
Salmonella typhimurium TA98
Salmonella typhimurium TA 1537

The study was a triple plate, independent repeat design. A preliminary toxicity study was conducted using five concentrations of test material from 50 to 5000 microgram per plate. The test material was determined to be cytotoxic to Salmonella at 500 micrograms per plate and above and cytotoxic to E coli at 1500 micrograms per plate and above.

Evaluation criteria were as follows: When the number of revertant colonies on the plate containing the test substance was found to be more than two times that of the negative control, and at the same time, when reproducibility or dose dependency for its increase is seen in more than one strain of bacteria by either the direct or metabolic activation method, the said test substance was determined to be mutagenic (positive) for those strains.

Result :

Only strains TA100 and TA98 showed increases in revertants and data are shown in this robust summary only for these two strains

The tables below show the mean of the revertants from three replicate plates

TA100	Trial 1		Trial 2	
Dose	-S9	+S9	-S9	+S9
0	129	134	123	121
19.53	96	140	141	130
39.06	98	154	193	183
78.12	116	165	338	369
156.2	199	238	228	264
312.5*	175	129	16	96
625*	2	19	0	0

TA98	Trial 1		Trial 2	
Dose	-S9	+S9	-S9	+S9
0	17	27	19	23
19.53	21	31	29	38
39.06	28	34	55	39
78.12	59	44	74	47
156.2	95	58	53	48
312.5*	38	33	0	10
625*	0	0	0	0

* = Bacterial growth inihibition

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Conclusion :

TA100 and TA98 showed numbers of revertants and dose dependency consistent with the evaluation criteria for a positive result. The test material is considered positive for mutagenic activity in this system under these conditions.

Reliability : (1) valid without restriction
Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

21.08.2003 (13)

Type : Chromosomal aberration test
System of testing : Chinese hamster lung cells
Test concentration : 0.005 to 0.032 mg/ml
Cycotoxic concentr. : 0.02 or 0.032 in the presence of S9 mix
Metabolic activation : with and without
Result : positive
Method :
Year :
GLP :
Test substance :

Method :

Frozen Chinese hamster lung (CHL) cells derived from Chinese hamsters (obtained February, 1988, in the fourth successive generation from Research Resource Bank (JCRB)) were thawed and used for the test within the tenth successive generation. Eagle MEM culture medium with 10% fetal calf serum was used as the growth media.

CHL cells (20,000) were seeded into 5 ml culture medium in a flask (Croning 25 cm2) and was incubated in a CO2 incubator (5% CO2) at 37?.

For the direct method, the test substance was added on the 3rd post-seeded

day and the samples were exposed to the test substance for either 24 or 48 hours. For metabolic activation with and without the presence of S9 mix, the samples were exposed for 6 hours on the 3rd post-seeded day and upon completion of the exposure they were further cultured in fresh media for an additional 18 hours.

Dilutions of test substance were freshly prepared in acetone before each use. Containers with caps were used to minimize any changes occurring from volatilization of the substance during the preparation and handling. The test substance was dissolved in the solvent and then further diluted acetone serially to obtain the desired concentrations of the test solution. The test solution was then added to the culture media at 0.5% (v/v) for all testing. Analytical measurements of the test substance in acetone dilutions were conducted and all concentration except the 1.00 mg/ml concentration were within the acceptable range (85% of the added amount). The deviation form target concentration in the 1.0 mg/ml dilution was attributed to volatility of the test material.

Cytotoxicity was determined by adding different concentrations of MM to the cultures using the direct, the indirect and the indirect with S-9 culture conditions. Growth inhibition was measured by determining the mitotic index. The concentration exerting 50% growth inhibition (50% reduction of mitotic index) was found to be 0.020 mg/ml for the direct method while the 50% inhibitory concentrations for metabolic activation with and without S-9 mix were 0.032 mg/ml and 0.019 mg/ml, respectively. The source of the S9 was not reported.

Dose selection: Based on the results from the cell growth inhibition test, the high concentrations of the test substance were determined to be 0.020 mg/ml for the direct method and 0.032 mg/ml and 0.020 mg/ml for the metabolic activation method with S9 mix and without S9 mix, respectively. Half strength of each corresponding high concentration was used as the medium concentration and 1/4 as the low concentration.

Two hours prior to the completion of incubation, Colcemid was added to the culture media so that its final concentration was approximately 0.1µg/ml. Six slides were prepared from each petri dish and were stained with 3% Giemza solution for 10 minutes.

Slides were coded and read blind. The chromosomal analysis was based on the classification by the Japan Environmental Mutagen Association, Mammalian Mutation Study (MMS) Subcommittee, and structural aberrations of chromosome or chromatid such as gaps, breaks and exchanges, as well as polyploid cells were scored. For structural aberrations, 200 cells per group and for poliploid cells, 800 metaphase cells per each group were analyzed.

Statistics analysis was conducted using Fisher's exact test to determine the significance of differences in the number of cells with chromosomal aberrations between the solvent control groups and the groups treated with the test substance, and the positive control groups. The potential of the test substance to induce chromosomal aberrations was determined based on the criteria established by Ishidate et al. where the percentage of cells with chromosomal aberrations less than 5% is considered negative, while a percentage of more than 5% and below 10% is considered equivocal and if greater than 10% it is considered positive.

Result :

Results of chromosomal analysis using the direct method are shown in Table 1. As the result of exposure to methoxymethanol for 24 hours, the percentage cells with chromosomal structural aberrations and polyploid cells increased significantly in a concentration dependent relation. Methoxymethanol was determined to be positive for structural aberrations. The evaluation of polyploid cells was equivocal. With the 48-hour exposure, chromosomal structural aberrations were induced in 6% of the cells (including gaps) in the high concentration group (0.020mg/ml) indicating an equivocal result. There were also significant increases in the number of polyploid cells in the low concentration group (0.005mg/ml) and in the high concentration group (0.020 mg/ml) indicating an equivocal result for the high concentration group.

Results of chromosomal analysis using metabolic activation are shown in Table 2. Following the application of methoxymethanol, the high concentration groups with 6-hour exposure with and without the presence of S9 mix revealed chromosomal aberrations (including gaps) in 16.5%- 26% of the studied cells indicating a positive result. Further, there was a significant increase in the appearance frequency of polyploid cells among the medium and high concentration groups indicating an equivocal result.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Attached document : CA Tab-1.bmp
CA Tab-2.bmp

Table 1 Chromosome analysis of Chinese hamster cells (CHL) continuously treated with methoxymethanol ** without S9 mix

Group	Concent-ration (mg/ml)	Time of exposure (hr)	No. of cells analysed	No. of structural aberrations: gap	ctb	cte	csb	cse	f	mul[2]	total	Others[3]	No. of cells with aberrations: TAG	(%)	TA	(%)	Polyploid[4] (%)	Judgement[5]: SA	NA
Control			200	0	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.25		
Solvent[1]	0	24	200	0	0	0	0	0	1	0	1	0	1	(0.5)	1	(0.5)	0.13		
MOM	0.005	24	200	0	0	0	0	1	0	0	1	3	1	(0.5)	1	(0.5)	0.13	−	−
MOM	0.010	24	200	0	3	14	0	0	0	0	17	1	10 *	(5.0)	10 *	(5.0)	3.13 *	±	−
MOM	0.020	24	200	1	29	74	1	2	1	0	108	3	41 *	(20.5)	40 *	(20.0)	5.88 *	+	±
MC	0.00005	24	200	3	25	50	3	4	0	0	85	1	59 *	(29.5)	57 *	(28.5)	0.13	+	−
Solvent[1]	0	48	200	0	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.13		
MOM	0.005	48	200	0	0	1	0	0	0	0	1	0	1	(0.5)	1	(0.5)	1.38 *	−	−
MOM	0.010	48	200	0	0	0	0	0	0	0	0	2	0	(0.0)	0	(0.0)	1.00	−	−
MOM	0.020	48	200	1	1	10	0	3	2	10	27	6	12 *	(6.0)	11 *	(5.5)	5.00 *	±	±
MC	0.00005	48	200	4	21	53	2	3	16	0	99	8	59 *	(29.5)	59 *	(29.5)	0.38	+	−

Abbreviations : gap : chromatid gap and chromosome gap, ctb : chromatid break, cte: chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring etc.), f : acentric fragment (chromatid type), mul : multiple aberrations, TAG : total no. of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, MC : mitomycin C.
1) Acetone was used as solvent. 2) More than ten aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Judgement was done on the basis of the criteria of Ishidate et al. (1987). * : Significantly different from solvent control at $p < 0.05$. ** : Purity was 46.73%, and methanol (44.93%) was contained as impurity

Table 2 Chromosome analysis of Chinese hamster cells (CHL) treated with methoxymethanol. ** with and without S9 mix

Group	Concentration (mg/ml)	S9 mix	Time of exposure (hr)	No. of cells analysed	No. of structural aberrations: gap	ctb	cte	csb	cse	f	mul[2]	total	Others[3]	No. of cells with aberrations: TAG	(%)	TA	(%)	Polyploid[4] (%)	Judgement[5]: SA	NA
Control				200	0	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	0.50		
Solvent[1]	0	−	6 - (18)	200	0	0	:	0	0	0	0	1	1	1	(0.5)	1	(0.5)	1.50		
MOM	0.005	−	6 - (18)	200	0	0	0	0	0	0	0	0	0	0	(0.0)	0	(0.0)	1.25	−	−
MOM	0.010	−	6 - (18)	200	0	1	2	0	0	0	0	3	0	2	(1.0)	2	(1.0)	3.25 *	−	−
MOM	0.020	−	6 - (18)	200	0	28	67	0	0	.	10	126	0	52 *	(26.0)	52 *	(26.0)	2.65 *	+	−
CPA	0.005	−	6 - (18)	200	2	0	:	0	0	0	0	3	1	3	(1.5)	1	(0.5)	0.13	−	−
Solvent[1]	0	+	6 (18)	200	0	0	:	0	0	0	0	1	1	1	(0.5)	1	(0.5)	0.25		
MOM	0.008	+	6 - (18)	200	1	0	:	0	0	0	0	2	1	2	(1.0)	1	(0.5)	0.13	−	−
MOM	0.016	+	6 - (18)	200	1	0	0	0	0	:	0	2	0	2	(1.0)	1	(0.5)	1.63 *	−	−
MOM	0.032	+	6 - (18)	200	2	16	41	0	1	2	0	62	2	33 *	(16.5)	32 *	(16.0)	5.75 *	+	±
CPA	0.005	+	6 (18)	200	4	32	33	2	0	3	0	64	4	49 *	(24.5)	45 *	(22.5)	0.13	+	−

Abbreviations : gap : chromatid gap and chromosome gap, ctb : chromatid break, cte : chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring etc.), f : acentric fragment (chromatid type), mul : multiple aberrations, TAG : total no. of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, CPA : cyclophosphamide.
1) Acetone was used as solvent. 2) More than ten aberrations in a cell were scored as 10 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group 5) Judgement was done on the basis of the criteria of Ishidate et al. (1987). 6) Seven hundred and nineteen-three cells were analysed. * : Significantly different from solvent control at $p < 0.05$ ** : Purity was 46.73%, and methanol (44.93%) was contained as impurity

Conclusion :
Under the conditions of this study, it is concluded that methoxymethanol induces chromosomal aberrations to CHL cells in vitro.

Reliability : (1) valid without restriction
Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

21.08.2003 (12)

5.6 GENETIC TOXICITY 'IN VIVO'

5.8.1 TOXICITY TO FERTILITY

Type : Fertility
Species : rat
Sex : male/female
Strain : Crj: CD(SD)
Route of admin. : gavage
Exposure period : 14 day premating to lactation day 4
Frequency of treatm. : daily
Premating exposure period
Male : 14 days
Female : 14 days
Duration of test :
No. of generation studies : 2
Doses : 12, 60 or 300 mg/lg-day
Control group : yes, concurrent vehicle
Method : OECD Guide-line 422
Year :
GLP : yes

Test substance : other TS: see freetext

Method :

Sprague-Dawley rats (Crj:CD, SPF) obtained from Charles River Laboratories, Japan were acclimated for six days before they were divided into groups of 10 animals of each sex using stratified random sampling by weight. Rats were 8 weeks old and their weight ranged from 278-309g for males and 186-215g for females at the first dosing.

The animal room used a 12-hour day light cycle and was regulated to maintain the temperature between 20-25° C, the humidity between 40-70% R.H., and ventilation at about 12 changes of air per hour. Animals were housed in polycarbonate boxes using bedding (Betachip: Charles River Laboratories, Japan). Except during breeding, when one male and one female were co-housed, animals were individually housed. After delivery, the dam and her litter were kept in the same cage during the lactation period.

Autoclaved feed (CRF-1: Oriental Yeast Co., Ltd.) and tap water that was filtered through a 5μm filter and was irradiated with ultraviolet rays were offered ad lib.

DOSE SELECTION: Dose levels of 0,12, 60 or 300 mg/kg-day were selected based on a preliminary study with dose levels of 0, 30, 100, 300 or 1000 mg/kg-day. The 1000 mg/kg-day group showed signs of overt toxicity including reduced spontaneous activity, irregular respiration, lacrimation and death. Necropsy revealed erosion or ulceration of the stomach or duodenum in the high-dose group. The 300 mg/kg-day group was reported to show salivation and changes in the stomach but these effects were considered a LOAEL and 300 mg/kg-day was selected as the high dose for the definitive study.

STUDY CONDUCT: Males were dosed for 44 days starting 14 days prior to mating and were sacrificed the day after the last dosing. Females were dosed for 41 to 47 days starting 14 days before mating, through mating and delivery, and three days of lactation. The test substance was diluted with distilled water prior to dosing and given by gavage as a single daily administration in the morning. Dosing volume was 5ml/kg calculated based on the most current body weight measured at that time.

Rats were mated one male and one female within the same group and allowed to mate for seven days. During this period, every day in the morning, the female's vaginal mucus was collected and was microscopically examined after it was Giemsa stained. Day zero of gestation was recorded when either a vaginal plug or sperm was found in the vaginal specimen.

Pregnant females were allowed to deliver their pups naturally. Lactation day zero was defined as completion of delivery by 9:00 in the morning of day zero. Pups were allowed to nurse until lactation day 4 and observed daily during this time for general condition, lactation, nesting, cannibalism and other significant signs. Surviving dams and pups were sacrificed on lactation-day 4. Ovaries and uteri of dams were removed to count corpora lutea and implantation sites. Based on the results obtained from these examinations, the gestation period, the gestation index, the implantation index and the delivery index were calculated.

EXAMINATION OF PUPS: Dead pups, except those that were killed and

eaten and unfit for examination, were fixed in a mixed solution of formaldehyde and acetic acid before being microscopically examined. Pups from each dam were separated by sex and weighed as a group of one sex on days zero and 4. External examinations, including the oral cavity, were conducted on lactation day 4. After the examination, about half of the pups from each litter were sacrificed and prepared for skeletal examination. Pups from the control group and the high-dose group were examined for skeletal abnormalities. Pups not selected for skeletal examination were submitted to visceral examinations after fixation with a mixture of formaldehyde and acetic acid. Heads from the control and high-dose groups were examined using Wilson's method and their chest and abdomen were micro-dissected to discover any visceral abnormalities. Since there was a slightly increased occurrence of patent foramen ovale in the 300 mg/kg-day group, the 60 mg/kg-day group was also examined for visceral abnormalities.

STATISTICAL METHODS:

Data were tested for homogeneity using Bartlett's method and when the distribution was normal, a one-way distribution dispersion analysis was performed. Then using either Dunnett's or Scheffe's test, the mean values were compared. When the distribution was not normal, the Kruskal-Wallis test was applied before the rank sum test of either Dunnett's or Scheffe's method. Some parameters (with asterisk) were tested initially using the Kruskal-Wallis test and when there was a significant difference, the rank sum test was performed. The calculated data were tested using Fisher's direct probability method. The level of significance was set to 5%. The mean values calculated from each maternal group were used as their statistical units for the data pertaining to the newborn pups. The following are the items for the statistical analysis.

Multiple comparison tests were used with: Weight, weight gain, feed consumption, hematological tests, blood biochemistry tests, weight of organs, paring days*, number of estrous cycles before successful copulation*, gestation period*, number of corpora lutea, number of implantation sites, implantation index*, delivery index*, number of newborn pups, weight of newborn pups, live birth index*, viability index*, and the occurrence of skeletal and visceral abnormalities among live pups*

Fisher's direct probability method was used with: Copulation index, fertility index, gestation index, and sex ratio (male/female)

Result :

DEATHS: One male from the 300 mg/kg-day group died on the 14th day of administration.

CLINICAL SIGNS: Slight salivation after administration of the test substance was observed in the 300 mg/kg-day group starting on the second administration day for males, and the fourth day for the females lasting and was observed for almost all animals. Some started salivating even before the dose was given and one male showed decreased spontaneous activities and gasping on the 13th day before dying the next day. One female was observed with rales starting on the 12th day of administration and lasting through the 6th day of gestation. A few males and females in the 60 mg/kg-day group also displayed salivation but this was a sporadic occurrence.

BODY WEIGHTS: Suppression of body weight gain was noted among males of the 300 mg/kg-day group from the 7th day of administration throughout the rest of the administration period. Females did not show any significant

difference between controls and dosed groups throughout the periods before mating, during gestation and after delivery.

FEED CONSUMPTION: Reduced feed consumption was noted for high dose males starting on the seventh day of dosing and continuing until sacrifice. Feed consumption for other dose groups was not different from controls before mating, during gestation period and after delivery.

HEMATOLOGY: A decrease in the red blood cell count, hematocrit value and hemoglobin concentration was noted for the high dose males as well as an increase in both reticulocyte and platelet counts. The leukocyte differential count was unremarkable for all dosed groups.

BIOCHEMISTRY: A decrease in the total protein, albumin and calcium and an increase in the A/G ratio were noted in the high-dose males. Chloride was also increased in the high-dose males but the increase was very slight and is not considered toxicologically significant.

ORGAN WEIGHTS: There was no significant difference in any of the organs between the control group and the dosed groups.

GROSS EXAMINATION: Either ulceration or erosion of the gastric glands and the proventriculus mucus membrane of the stomach were noted in 3 males and 2 females in the 300 mg/kg-day group. Five males and 4 females in the high-dose group showed the formation of gastric nodules in various sizes. Six high-dose males showed an enlarged duodenum. One high-dose male showed enlarged adrenal glands. The high-dose male that died on test had an enlarged atrium, pulmonary congestion, atrophy of the thymus gland, red patches in the gastric gland mucosa and distension of the bowel.

MICROSCOPIC EXAMINATION: Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300 mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border.

Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls.

Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

REPRODUCTIVE TOX: All females that copulated resulted in pregnancy and no effect of the administered test substance on either the copulation or fertility indices was recognized. Further, most of the pairs successfully mated during the first estrous stage and there were no significant differences among the pairing days. Also, no histopathological changes were found in the ova of the single female of which copulation was unconfirmed. Reproductive parameters are shown in the table.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Attached document : Rerpo.bmp

Table 6 Summary of reproductive performance in rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

Dose level	0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
No. of animals	10	10	10	10
No. of pairs copulated	10	10	10	10
No. of pregnant females	10	10	10	10
Copulation index (%) [a]	100.0	90.0	100.0	100.0
Fertility index (%) [b]	100.0	100.0	100.0	100.0
Pairing days [c]	2.8 ± 1.48	2.4 ± 1.01	3.2 ± 1.62	2.8 ± 1.32
E.S. [d]	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.32	0.0 ± 0.00
(Mean ± S.D.)				

a) (Number of animals with successful copulation/number of animals mated)×100
b) (Number of pregnant animals/number of animals with successful copulation)×100
c) Days between initial pairing and detection of copulation.
d) Number of estrous stages without copulation.

Conclusion :

No adverse effects were seen on reproduction in this screening study.

Reproductive NOAEL 300 mg/kg-day

Parental NOAEL
12 mg/kg-day (males)
60 mg/kg-day (females)

Reliability : (1) valid without restriction

Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

01.12.2003 (9)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	14 days premating to lactation day 4
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	12, 60 or 300 mg/kg bw-day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 60 mg/kg bw
NOAEL teratogen.	:	= 300 mg/kg bw
NOAEL Fetotoxicity	:	= 60 mg/kg bw
Result	:	Not specific developmental toxin
Method	:	other: OECD Guideline 422
Year	:	
GLP	:	yes
Test substance	:	other TS: see freetext

Method :

Sprague-Dawley rats (Crj:CD, SPF) obtained from Charles River Laboratories, Japan were acclimated for six days before they were divided into groups of 10 animals of each sex using stratified random sampling by weight. Rats were 8 weeks old and their weight ranged from 278-309g for males and 186-215g for females at the first dosing.

The animal room used a 12-hour day light cycle and was regulated to maintain the temperature between 20-25° C, the humidity between 40-70% R.H., and ventilation at about 12 changes of air per hour. Animals were housed in polycarbonate boxes using bedding (Betachip: Charles River Laboratories, Japan). Except during breeding, when one male and one female were co-housed, animals were individually housed. After delivery, the dam and her litter were kept in the same cage during the lactation period.

Autoclaved feed (CRF-1: Oriental Yeast Co., Ltd.) and tap water that was filtered through a 5μm filter and was irradiated with ultraviolet rays were offered ad lib.

DOSE SELECTION: Dose levels of 0,12, 60 or 300 mg/kg-day were selected based on a preliminary study with dose levels of 0, 30, 100, 300 or 1000 mg/kg-day. The 1000 mg/kg-day group showed signs of overt toxicity including reduced spontaneous activity, irregular respiration, lacrimation and death. Necropsy revealed erosion or ulceration of the stomach or duodenum in the high-dose group. The 300 mg/kg-day group was reported to show salivation and changes in the stomach but these effects were considered a LOAEL and 300 mg/kg-day was selected as the high dose for the definitive study.

STUDY CONDUCT: Males were dosed for 44 days starting 14 days prior to mating and were sacrificed the day after the last dosing. Females were dosed for 41 to 47 days starting 14 days before mating, through mating and delivery, and three days of lactation. The test substance was diluted with distilled water prior to dosing and given by gavage as a single daily administration in the morning. Dosing volume was 5ml/kg calculated based on the most current body weight measured at that time.

Rats were mated one male and one female within the same group and allowed to mate for seven days. During this period, every day in the morning, the female's vaginal mucus was collected and was microscopically examined after it was Giemsa stained. Day zero of gestation was recorded when either a vaginal plug or sperm was found in the vaginal specimen.

Pregnant females were allowed to deliver their pups naturally. Lactation day zero was defined as completion of delivery by 9:00 in the morning of day zero. Pups were allowed to nurse until lactation day 4 and observed daily during this time for general condition, lactation, nesting, cannibalism and other significant signs. Surviving dams and pups were sacrificed on lactation-day 4. Ovaries and uteri of dams were removed to count corpora lutea and implantation sites. Based on the results obtained from these examinations, the gestation period, the gestation index, the implantation index and the delivery index were calculated.

EXAMINATION OF PUPS: Dead pups, except those that were killed and eaten and unfit for examination, were fixed in a mixed solution of formaldehyde and acetic acid before being microscopically examined. Pups from each dam were separated by sex and weighed as a group of one sex on days zero and 4. External examinations, including the oral cavity, were conducted on lactation day 4. After the examination, about half of the pups from each litter were sacrificed and prepared for skeletal examination. Pups from the control group and the high-dose group were examined for skeletal abnormalities. Pups not selected for skeletal examination were submitted to visceral examinations after fixation with a mixture of formaldehyde and acetic acid. Heads from the control and high-dose groups were examined using Wilson's method and their chest and abdomen were micro-dissected to discover any visceral abnormalities. Since there was a slightly increased occurrence of patent foramen ovale in the 300 mg/kg-day group, the 60 mg/kg-day group was also examined for visceral abnormalities.

STATISTICAL METHODS: Data were tested for homogeneity using Bartlett's method and when the distribution was normal, a one-way distribution dispersion analysis was performed. Then using either Dunnett's or Scheffe's test, the mean values were compared. When the distribution was not normal, the Kruskal-Wallis test was applied before the rank sum test of either Dunnett's or Scheffe's method. Some parameters (with asterisk) were tested initially using the Kruskal-Wallis test and when there was a significant difference, the rank sum test was performed. The calculated data were tested using Fisher's direct probability method. The level of significance was set to 5%. The mean values calculated from each maternal group were used as their statistical units for the data pertaining to the newborn pups. The following are the items for the statistical analysis.

Multiple comparison tests were used with: Weight, weight gain, feed consumption, hematological tests, blood biochemistry tests, weight of organs, paring days*, number of estrous cycles before successful copulation*, gestation period*, number of corpora lutea, number of implantation sites, implantation index*, delivery index*, number of newborn pups, weight of newborn pups, live birth index*, viability index*, and the occurrence of skeletal and visceral abnormalities among live pups*

Fisher's direct probability method was used with: Copulation index, fertility index, gestation index, and sex ratio (male/female)

Result :

DEATHS: One male from the 300 mg/kg-day group died on the 14th day of administration.

CLINICAL SIGNS: Slight salivation after administration of the test substance was observed in the 300 mg/kg-day group starting on the second administration day for males, and the fourth day for the females lasting and was observed for almost all animals. Some started salivating even before the dose was given and one male showed decreased spontaneous activities and gasping on the 13th day before dying the next day. One female was observed with rales starting on the 12th day of administration and lasting through the 6th day of gestation. A few males and females in the 60 mg/kg-day group also displayed salivation but this was a sporadic occurrence.

BODY WEIGHTS: Suppression of body weight gain was noted among males of the 300 mg/kg-day group from the 7th day of administration throughout the rest of the administration period. Females did not show any significant difference between controls and dosed groups throughout the periods before mating, during gestation and after delivery.

FEED CONSUMPTION: Reduced feed consumption was noted for high dose males starting on the seventh day of dosing and continuing until sacrifice. Feed consumption for other dose groups was not different from controls before mating, during gestation period and after delivery.

HEMATOLOGY: A decrease in the red blood cell count, hematocrit value and hemoglobin concentration was noted for the high dose males as well as an increase in both reticulocyte and platelet counts. The leukocyte differential count was unremarkable for all dosed groups.

BIOCHEMISTRY: A decrease in the total protein, albumin and calcium and an increase in the A/G ratio were noted in the high-dose males. Chloride was also increased in the high-dose males but the increase was very slight and is not considered toxicologically significant.

ORGAN WEIGHTS: There was no significant difference in any of the organs between the control group and the dosed groups.

GROSS EXAMINATION: Either ulceration or erosion of the gastric glands and the proventriculus mucus membrane of the stomach were noted in 3 males and 2 females in the 300 mg/kg-day group. Five males and 4 females in the high-dose group showed the formation of gastric nodules in various sizes. Six high-dose males showed an enlarged duodenum. One high-dose male showed enlarged adrenal glands. The high-dose male that died on test had an enlarged atrium, pulmonary congestion, atrophy of the thymus gland, red patches in the gastric gland mucosa and distension of the bowel.

MICROSCOPIC EXAMINATION: Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300

mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border.

Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls.

Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

DEVELOPMENTAL TOX

VIABILITY: A few still births and neonatal deaths occurred in each group, but there was no significant difference between the control group and dose groups regarding the number of pups in the litter, number of live pups, sex ratio, or live birth and viability indices.

EXTERNAL EXAMINATION: No newborn pups showed any external abnormalities in any group and their general condition subsequent to their birth indicated no abnormalities attributable to the administered test substance.

PUP WEIGHTS AND WEIGH GAIN: For both males and females, the weights measured on the lactation days 0 and 4, and the weight increase between these two dates showed no significant difference between the control group and the dose groups.

SKELETAL EXAMINATION: There were no skeletal malformations found in the control or 300 mg/kg-day groups. As variations, excess hypoglossal foramen, closure of the transverse foramen of cervical vertebrae, splitting of the ossification center of vertebral tubercle of the atlas, accessory sternebra, cervical rib, 14th rib (costal vestigium) and a shortening of the 13th rib were noted. These variations were not significantly increased as compared to the control group. Further, the occurrence of accessory sternebra in the 300mg/kg-day group was marginally significant and was considered an incidental finding.

VISCERAL EXAMINATION: There was a significant increase in the occurrence of patent foramen ovale in the 300 mg/kg-day group. In the 300 mg/kg-day group, the incidence was 10 pups from 6 litters. Control incidence was 2 pups from 2 litters. One pup form the 60 mg/kg-day group displayed this pathology. Other findings were not dose related and were considered incidental.

VISCERAL EXAMINATION OF DEAD PUPS: The number of early-death pups that were suitable for examination was 1, 3, 2, and 9 pups from the

control, 12 mg/kg-day, 60 mg/kg-day, and the 300 mg/kg-day group, respectively. Among pups found dead on the day of delivery, one high-dose pup had a hydrocephalus. Among those that expired after lactation day 1, one pup each from the control group and the high-dose group showed patent ductus arteriosus, and one pup from the 12 mg/kg group revealed dilatation of the renal pelvis. As there were few findings and no dose-response relationship these effects are considered unrelated to administration of the test substance. Other findings from the animals that died on the day of delivery include, patent foramen ovale was found in one pup from the 12 mg/kg-day group and in 2 pups from the 300 mg/kg-day group. There were also 4 cases of patent ductus arteriosus in the 300 mg/kg-day group. These findings are attributed to the fact that the pups died during parturition resulting in an incomplete closure of either the foramen ovale or ductus arteriosus.

Test substance :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

Attached document : Develop-Finds.bmp
Develop.bmp

Table 8 Skeletal and visceral findings of pups (F1)from dams (F0)treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

Dose level	0 mg/kg		60 mg/kg		300 mg/kg	
No. of dams	10		10		10	
Skeletal examination						
No. of pups examined	78		$		77	
No. of abnormal pups (%)	15	(18.9)			13	(16.3)
Foramen hypoglossi double	1	(1.3)			0	
Closure of transverse foramen of one or more cervical vertebrae	4	(5.1)			9	(11.1)
Splitting of ossification centers of the ventral tubercle of the atlas	0				1	(1.3)
Accessory sternebrae	5	(6.2)			0	**
Cervical ribs	0				1	(1.4)
14th ribs	2	(2.5)			0	
Reduced 13th ribs	4	(5.0)			2	(2.5)
Visceral examination						
No. of pups examined	72		62		72	
No. of abnormal pups (%)	8	(10.9)	8	(12.2)	16	(22.4)
Thymic remunant in the neck	3	(4.1)	2	(3.3)	3	(4.2)
Deformity of the heart	1	(1.4)	0		0	
Patent foramen ovale	2	(2.7)	1	(1.7)	10	(14.5)*
Patent ductus arteriosus	2	(2.7)	2	(2.9)	3	(3.8)
Supernumerary of the comary orifice	2	(2.7)	0		0	
High take off of the comary orifice	0		0		1	(1.3)
Dilatation of the renal pelvis	0		3	(4.3)**	0	

$: Not examined.
Significantly different from control group; *: P<0.05, **: P<0.01.

Table 7 Findings of delivery in dams (F0) treated orally with methoxymethanol and observation on their pups (F1) in combined repeat dose and reproductive/developmental toxicity screening test

Dose level			0 mg/kg	12 mg/kg	60 mg/kg	300 mg/kg
No. of dams observed			10	9	10	10
No. of dams observed live pups			10	9	10	10
Gestation length			22.7 ± 0.48	22.4 ± 0.73	22.4 ± 0.52	22.2 ± 0.42
No. of corpora lutea			18.1 ± 2.64	17.9 ± 3.92	18.2 ± 4.54	19.3 ± 4.00
No. of implantation sites			16.4 ± 1.35	14.3 ± 4.92	14.6 ± 3.24	16.4 ± 1.84
No. of pups born			15.2 ± 1.55	14.2 ± 5.07	13.6 ± 3.20	15.9 ± 1.73
No. of live pups on day 0			15.2 ± 1.55	14.1 ± 5.04	13.5 ± 3.10	15.2 ± 1.75
Male			7.0 ± 2.40	7.1 ± 3.59	5.7 ± 2.36	6.9 ± 2.33
Female			8.2 ± 2.94	7.0 ± 3.57	7.8 ± 2.53	8.3 ± 2.21
Sex ratio (Male/Female)			0.85 (70/82)	1.02 (64/63)	0.73 (57/78)	0.85 (69/83)
No. of live pups on day 4			15.0 ± 1.49	13.5 ± 5.34	13.1 ± 2.81	14.9 ± 1.66
Male			6.9 ± 2.51	6.9 ± 3.92	5.6 ± 2.22	6.8 ± 2.15
Female			8.1 ± 2.96	6.7 ± 3.16	7.5 ± 2.32	8.1 ± 2.02
Gestation index (%) [a]			100	100	100	100
Implantation index (%) [b]			91.6 ± 9.86	80.4 ± 24.98	81.9 ± 18.70	87.7 ± 16.05
Delivery index (%) [c]			92.7 ± 5.37	91.8 ± 16.20	93.0 ± 7.03	97.1 ± 4.00
Live birth index (%) [d]			100.0 ± 0.00	99.3 ± 2.07	99.4 ± 1.87	95.8 ± 6.35
Viability index (%) [e]			98.7 ± 2.66	86.1 ± 33.33	97.5 ± 4.36	98.1 ± 3.06
Pups body weight						
Male	On day	0	6.9 ± 0.54	6.4 ± 0.55	6.9 ± 0.94	6.2 ± 0.59
		4	11.1 ± 0.99	10.5 ± 0.84	10.9 ± 2.25	10.0 ± 0.85
	Gain	0-4	4.1 ± 0.59	4.1 ± 0.48	4.0 ± 1.45	3.8 ± 0.43
Female	On day	0	6.5 ± 0.60	6.0 ± 0.77	6.6 ± 0.87	5.9 ± 0.47
		4	10.6 ± 0.95	9.9 ± 1.15	10.7 ± 2.04	9.5 ± 0.93
	Gain	0-4	4.1 ± 0.54	3.9 ± 0.50	4.0 ± 1.25	3.6 ± 0.58

a) (Number of females with live pups/number of pregnant females) ×100
b) (Number of total implants/number of total corpora lutea) ×100
c) (Number of total pups/number of total implants) ×100
d) (Number of total live pups on day 0 after birth/number of total pups born) ×100
e) (Number of total live pups on day 4 after birth/number of total live pups on day 0) ×100

Conclusion :

No malformations were observed that were attributable to administration of the test substance. High-dose pups were not different from controls in body weight, sex ratio, mean pup weights, number of pups born, or other similar parameters. Visceral examination revealed a significant increase in the occurrence of patent foramen ovale in the 300 mg/kg-day group. This is interpreted as a fetotoxic effect at the high dose associated with a developmental delay.

Developmental NOAEL 60 mg/kg-day

Maternal NOAEL 60 mg/kg-day

Reliability : (1) valid without restriction

Guideline or guideline-like study with good documentation

Flag : Critical study for SIDS endpoint

01.12.2003 (9)

(1) Bills, D. et al.: "Investigation in fish control. 73. Formalin, its toxicity to nontarget aquatic organisms, persistence and counteraction"; Washington DC, U.S. Department of the Interior, Fish and Wildlife Service, 1-7,(1977)

(2) Bringmann,G., Kuehn,R., Vom Wasser 50, 45-60, 1978

(3) Calculation using EPIWIN 3.05 by Toxicology and Regulatory Affairs, October 2003

(4) Calculations and Estimate by Toxicology and Regulatory Affairs, Freeburg IL, using the EPA ECOSAR v0.99f program, November 2003.

(5) Calculations and Estimate by Toxicology and Regulatory Affairs, Freeburg IL, using the EPA ECOSAR v0.99f program, October 2003.

(6) Calculations by Toxicology and Regulatory Affairs, Freeburg IL, using the SRC EPIWIN 3.05 program, October 2003.

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